The Journal of
CLINICAL ENDOCRINOLOGY
AND METABOLISM

VOLUME 13 FEBRUARY, 1953 NUMBER 2
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INFLUENCE OF CHORIONIC GONADOTROPIN ON HUMAN OVARIAN HILUS CELLS (LEYDIG-LIKE CELLS)*

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THE effects of stimulation of testicular Leydig cells by chorionic gonadotropin are well known. Since the ovarian hilus cells are morphologically identical with and probably functionally related to Leydig cells, they might also be expected to respond to chorionic gonadotropin. Morphologic changes in response to such hormonal stimulation would suggest that the cells are functionally active, and not merely embryologic rudiments. Furthermore, the character of the cytologic response might reaffirm the kinship of the ovarian cells to testicular Leydig cells.

We have, therefore, attempted a histologic study of the ovarian hilus cells in a group of patients, following experimental administration of chorionic gonadotropin and have also studied a series of ovaries from pregnant women in whom the level of endogenous chorionic gonadotropin would, of course, be elevated.

INTRODUCTION AND BACKGROUND

Certain cells known as ovarian hilus cells, which are morphologically identical with the Leydig cells of the testis, are normally present in the ovarian hilus and mesovarium of women. Their significance in the normal

Received for publication June 21, 1952.

* This study was made possible by a grant-in-aid from Ayerst, McKenna and Harrison, Ltd.

hormonal economy is not clear but there is convincing evidence that they are capable of producing androgens. One of us (1) recently published a morphologic study of these cells with a survey of the pertinent literature and reported several cases in which masculinization was associated with hilus cell tumors and hyperplasias. Excellent histologic studies have been published by Berger (2–4) and Kohn (5) and, more recently, by Campenhout (6, 7).

Summary of morphologic data. The ovarian hilus cells are present in microscopic nests along the length of the ovarian hilus and adjacent mesovarium. The great variation both in number and location of the cells in the ovaries of normal women renders quantitative studies difficult. However, with multiple sections through the hilus and mesovarium, microscopic clusters of these cells are found in virtually all adult ovaries.

There is a fairly constant relationship between ovarian hilus cells and the abundant nonmyelinated nerves of the mesovarium. The cell nests are usually found not only in contiguity with nonmyelinated nerves, but often in clusters and singly within the nerve, separating individual nerve fibers. The significance of this curious relationship to nerve is obscure, but it is worth noting that identical histologic configurations are also seen in the male gonad, being present in the tunica albuginea, the hilus of the testis, and occasionally within the testis proper. The ovarian hilus cells also show an intimate relationship to the abundant blood vessels and lymphatics of the mesovarium.

The cytology of the ovarian hilus cell is identical with that of the testicular Leydig cell. The diversity of cell forms seen in Leydig cells is reproduced precisely in the ovarian hilus cells. Nuclear and cytoplasmic detail as well as distribution of lipid is identical in the two cells. Brown lipochrome pigment may be seen in both, as well as the characteristic perinuclear distribution of acidophilic granules and peripheral lipid vacuoles. Most significant, for the specific identification of these cells, are the occasional crystalloids of Reinke (Fig. 1). These rod-like, acidophilic inclusions are seen in the cytoplasm of testicular Leydig cells and ovarian hilus cells, but have been described in no other cells of the body. Crystalloids are present only in a small percentage of the cells but when present they serve as specific identification tags, distinguishing hilus and Leydig cells from morphologically similar cells such as lutein and adrenal cortical cells. In most tumors of ovarian hilus cells which we have studied, the crystalloids have been present and have served to verify the identity of the neoplasms.

We have personally encountered 5 cases of tumors of ovarian hilus cells (2 of which have been reported in detail) and 6 examples of hyperplasia of ovarian hilus cells, all associated with masculinizing syndromes. Recent reports of similar masculinizing tumors have appeared in the literature

(8, 9). These observations, together with the cytologic identity with Leydig cells, indicate the androgenic function of hilus cells. Furthermore, the presence of these cells in newborn babies, their prompt disappearance and subsequent reappearance at the time of puberty, their prominence in pregnancy and to a lesser extent at the time of the menopause, as well as their general cytologic characteristics as secreting cells, all suggest hormonal activity.

FIG. 1. Normal ovarian hilus cells with unusually conspicuous crystalloids of Reinke, in surgical specimen removed from a 39-year-old woman operated upon for uterine leiomyomas. ×500

MATERIAL AND METHODS

The present study is based on histologic examination of ovaries of pregnant women and of patients treated experimentally with chorionic gonadotropin. In the experimental group, 6 women who had regular menstrual cycles and normal secretory activity of the endometrium as determined by control biopsy, were given chorionic gonadotropin[1] intramuscularly in daily doses of 10,000 I.U. Their ages ranged from 22 to 39 years. The initial dose was begun early in the postovulation period. With this dosage, the onset of the next menstrual period was delayed and a decidual reaction identical with that of early pregnancy developed in all patients. The detailed findings including the results of the hormonal assays have been re-

[1] Kindly supplied by Dr. E. C. Reifenstein, Jr., Ayerst, McKenna and Harrison, Ltd.

ported separately (10). The present study is concerned with morphologic observations on the hilus cells in ovaries removed from 4 of the 6 patients. We are also indebted to Drs. Brown and Bradbury for permitting us to examine the ovary of one of their patients similarly treated, thus making a total of 5 patients whose ovaries were available for study. The period of hormone administration prior to oophorectomy ranged between twelve and twenty-one days in these patients.

It was, of course, not feasible to obtain ovarian hilus tissue from the same patients prior to hormone treatment, so that evaluation of changes in the hilus cells was of necessity based on a comparison with a group of over 50 control ovaries from patients of comparable age. Tissues were fixed in 10 per cent formalin or in Bouin's solution. Sections were stained with hematoxylin and eosin and by Masson's trichrome method. From each ovary, six to twelve blocks were cut to include the ovarian hilus and mesovarium, and multiple sections were cut from each block.

The ovaries from 17 pregnant patients and from 1 patient with metastasizing uterine chorioepithelioma were examined in similar fashion. Nine ovaries from patients in the first trimester of gestation were obtained as a result of operations for tubal pregnancy. Specimens were obtained from 3 patients during the middle trimester (4, 5 and 6 months, respectively). Two of these patients were operated upon for ovarian malignancy and a third died postoperatively after removal of a large pedunculated leiomyoma of the uterus. Ovaries from 5 women who died at term were obtained from autopsy material. Two of these patients died of amniotic fluid embolism, 1 of eclampsia eight days post partum, 1 of a ruptured uterus, and 1 during anesthesia for manual removal of the placenta.

RESULTS

Hilus cell changes following administration of chorionic gonadotropin. The ovarian hilus cells of patients who had received chorionic gonadotropin showed significant cytologic changes as compared with the controls. Most of the cells were larger than those of the controls and contained a more abundant granular, acidophilic cytoplasm, with only sparse lipid vacuolization—characteristics which are customarily associated with vigorous activity in testicular Leydig cells. Many cells showed the perinuclear zone of cytoplasmic granules and the peripheral zone of vacuolization characteristic of well developed Leydig cells (Fig. 2).

Mitotic figures were found in 2 instances (Fig. 3). In 1 patient, treated for twelve days with chorionic gonadotropin, several mitotic figures were noted among the hilus cells. In another, treated for twenty-two days, only a single mitotic figure was found. The presence of even these few mitoses is considered significant, since not a single mitosis was found in comparable

studies of the hilus cells of the 50 control ovaries. In fact, previous studies covering several hundred ovaries had never before revealed mitoses in hilus cells, with the exception of a few seen in hilus-cell tumors. Similarly, testicular Leydig cells rarely contain mitotic figures. Histologic studies such as those of Hooker (11) and others (12) suggest that, under stimulation, testicular Leydig cells differentiate from precursor cells indistinguishable from fibroblasts. Some authors, in fact, deny that mitotic figures occur in differentiated Leydig cells, although Maximow (13) has found them

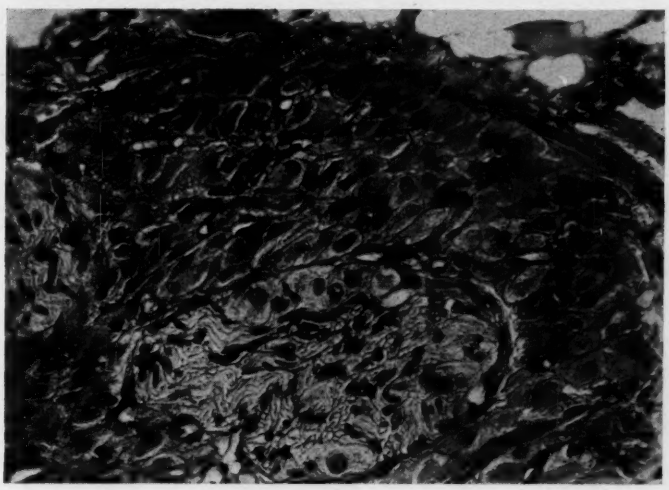

FIG. 2. Ovarian hilus cells following stimulation by chorionic gonadotropin, 10,000 I.U. daily for eighteen days, in a 22-year-old woman. Note the well developed cells, with abundant cytoplasm, and the perinuclear arrangement of cytoplasmic granules with vacuolization at the cell periphery. The usual relationship to nonmyelinated nerve is evident, and some hilus cells lie within the nerve. ×250

following aseptic trauma to the testis. The occurrence of mitotic figures in 2 of the 5 cases is probably not coincidental and is most likely the result of stimulation by chorionic gonadotropin.

In 3 of the 5 cases, areas of degeneration were present in occasional clusters of hilus cells. In these areas the cell outlines were lost, the cytoplasmic masses were dark, granular and smudged, and the nuclei misshapen, pyknotic and often clumped in dense aggregates (Fig. 4). Comparable foci were not found in the controls. We are uncertain as to the significance of these regions of degeneration. Possibly they represent an exhaustion effect associated with intense stimulation. The dosage of chorionic gonadotropin employed, although not excessive in terms of the amounts

produced in pregnancy, is nevertheless high when compared with that customarily employed clinically to produce stimulation of testicular Leydig cells.

It was not possible to establish that there was a definite increase in the number of hilus cells in the treated patients. Quantitative determination of

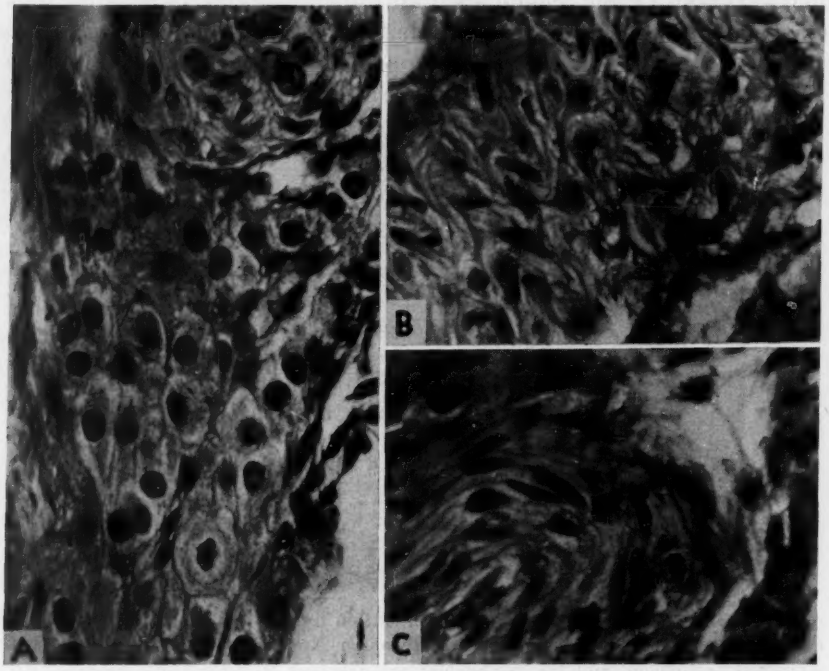

FIG. 3. Mitotic figures in ovarian hilus cells following stimulation by chorionic gonadotropin, experimentally administered. A and B from same patient as in Figure 2. C from another patient similarly treated. B and C illustrate mitoses in hilus cells within nonmyelinated nerve.

the number of cells present is extremely difficult. The hilus cells are widely dispersed in clusters of varying sizes throughout a considerable volume of tissue. Cell counts would be a formidable undertaking requiring serial sections of a large mass of tissue. Furthermore, there is considerable variation in the number of cells present in presumably normal ovaries from patients of comparable ages. It was estimated that the number of hilus cells was greater than average in 3 of the 5 treated patients, and average in the remaining 2. In all 5, however, the number fell within the possible range of normal.

Hilus cell changes in pregnancy and in chorioepithelioma. Nine ovaries

from patients with ectopic tubal pregnancies in the first trimester showed histologic changes in the hilus cells comparable with those described in the experimental group. The cells were large and the cytoplasm was extremely acidophilic and granular, with sparse vacuolization. In 2 instances there was a greater degree of nuclear and cytoplasmic pleomorphism than seen in the experimental cases. No mitoses or foci of degenerative change were

FIG. 4. Ovarian hilus cells, showing focal area of degenerative change following chorionic gonadotropin administration. Note dark acidophilic, nonvacuolated cytoplasm, loss of cell borders, and nuclear pyknosis and clumping. Nonmyelinated nerve tissue is seen in the upper and lower portions. ×390

seen, however. Crystalloids of Reinke were prominent in the hilus cells of 3 patients (Figs. 5 and 6).

The most striking changes in the hilus cells were seen in the term pregnancies and in the case of the fatal chorioepithelioma. In all 6 of these, the hilus cells were definitely hyperplastic. Large aggregates of hilus cells were seen in most sections examined (Fig. 7). Their numbers were estimated as several times the average in the controls. Cytologic changes were as prominent as the quantitative increase in cells. The cells were, on the average, much larger than those in the control ovaries, many reaching a diameter of over 40 micra, which is twice the average diameter of the normal hilus cell. The increase in size of the cell was largely the result of an increase in cytoplasm. The typical cell was large and polygonal with the nucleus compressed near one border. The cytoplasm in the majority of the

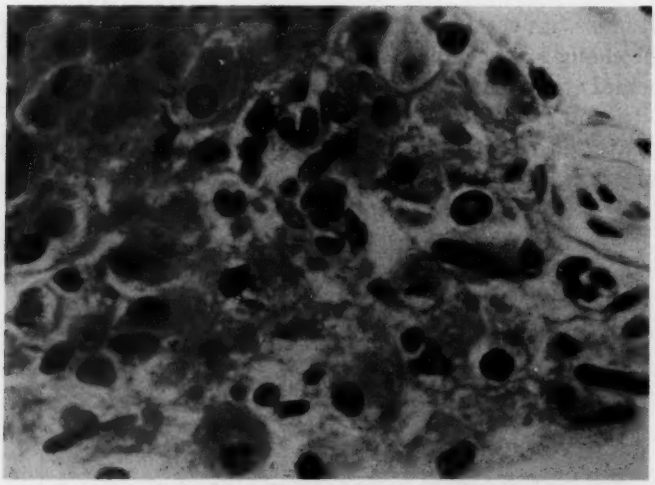

FIG. 5. Ovarian hilus cells in specimen from patient with ectopic tubal pregnancy (first trimester). Abundant large crystalloids of Reinke are present. ×630

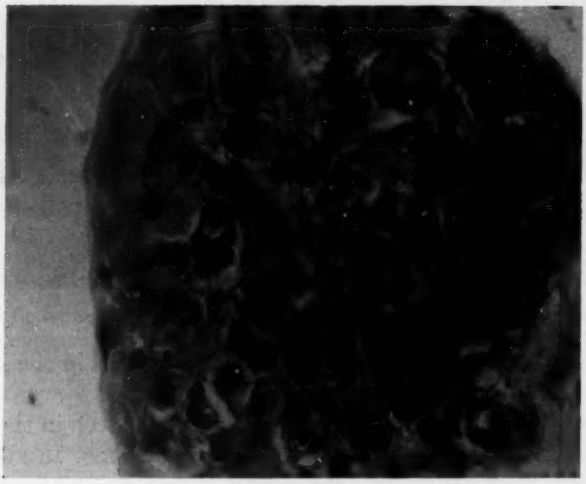

FIG. 6. Ovarian hilus cells from another example of ectopic tubal pregnancy (first trimester), showing large cells with granular cytoplasm. Abundant crystalloids of Reinke of the smaller variety are present. ×440

cells was strongly acidophilic and granular (Fig. 8). Nuclei were larger than in the controls, and showed greater variability, with larger chromatin clumps and denser staining. There was much greater pleomorphism than is usually seen among normal hilus cells. In 2 cases, large multinucleated

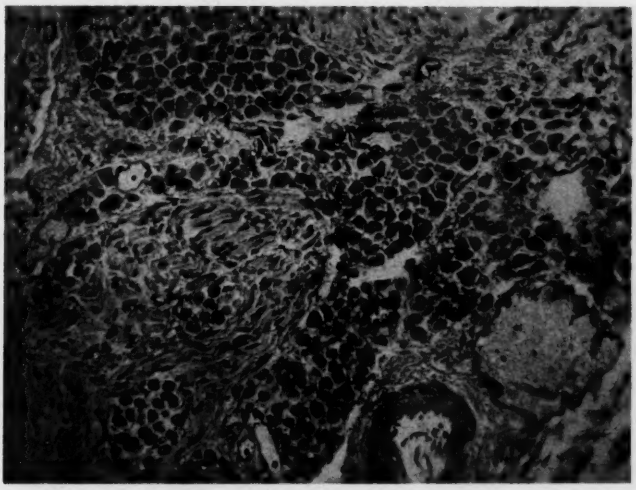

FIG. 7. Low-power view of hyperplastic ovarian hilus cells from patient at term, who died of amniotic fluid embolism. The cytoplasm is abundant, extremely acidophilic, and the nuclei are eccentrically placed at the cell borders. Note clusters of hilus cells within nerve trunk. ×140

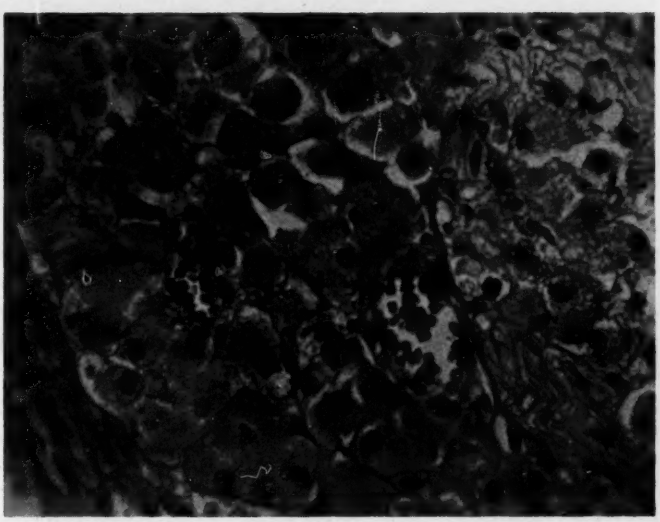

FIG. 8. Large, hyperplastic ovarian hilus cells at term (same patient as in Fig. 7). Note the abundant, extremely granular cytoplasm. There is a nonmyelinated nerve with hilus cells at the right. ×300

ovarian hilus cells were observed, which closely resembled the multinucleated Leydig cells occasionally seen in the testis (14). Pleomorphism was most pronounced in the case of postpartum eclampsia (Fig. 9). The specimen was obtained at autopsy eight days post partum and the greater cellular variation may have resulted from postpartum diminution of chorionic gonadotropin following the prolonged stimulation of pregnancy. In the patient who died at term with a ruptured uterus, a yellow nodule 1 mm. in diameter was grossly visible in the ovarian hilus. On section, this proved to be an adenoma-like mass of large hilus cells.

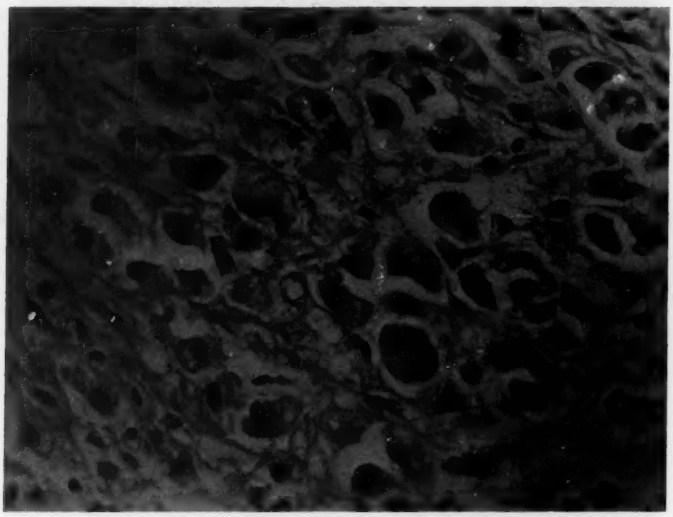

FIG. 9. Pronounced pleomorphism of ovarian hilus cells in patient who died of postpartum eclampsia eight days after delivery. ×300

The ovaries from the patient with chorioepithelioma also showed striking pleomorphism of the hilus cells. This 18-year-old patient died with multiple metastases, nine months after the birth of her child. Other evidences of the stimulation of the ovary by chorionic gonadotropin were the decidual reaction of the surface of the ovary and conspicuous theca luteinization. Two cytologic patterns were apparent in the hilus cells in this case: 1) large, moderately vacuolated, pale cells with large vesicular nuclei, and 2) small, darkly acidophilic, granular cells, deficient in vacuoles, with indistinct cell membranes and small, dark-staining, nonvesicular nuclei (Fig. 10). These differences in cellular pattern may well reflect different stages of cellular activity, secretion or exhaustion. It is interesting that these different cellular patterns were not casually intermingled. Rather, a single aggregate of cells would show one uniform pattern, while adjacent

aggregates would show a dissimilar pattern. Apparently, the cells in a single cluster were in the same stage of cellular activity, but were out of phase with the cells in adjacent groups. Comparable patterns were seen in the ovaries from several term pregnancies.

The ovarian hilus cells from patients in the middle trimester of pregnancy were similar to those at term, although the increase in number and the degree of cellular variation were not as conspicuous. In general, the advance of pregnancy to term was reflected in a progressive increase in numbers, size, and pleomorphism of the ovarian hilus cells.

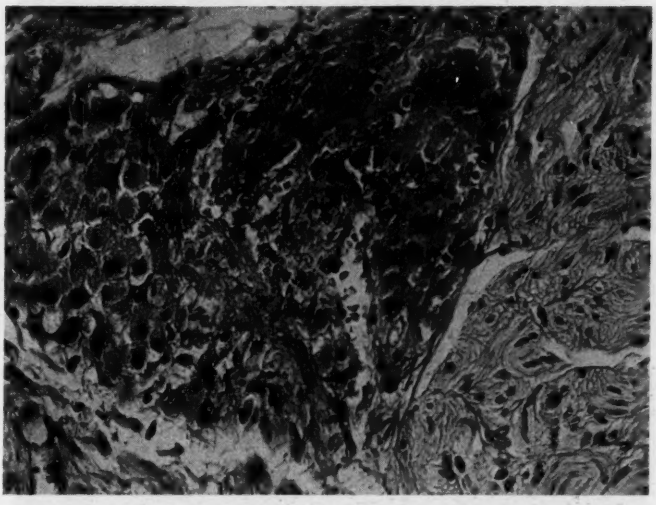

FIG. 10. Hyperplastic ovarian hilus cells from patient dying from metastatic chorioepithelioma. Small, dark, hilus cells with pyknotic nuclei are seen at the right of center; larger, more vacuolated cells at the left. There is a nonmyelinated nerve at the far right. ×180

Multiplication and differentiation of ovarian hilus cells. From the foregoing observations, it appears that chorionic gonadotropin causes an increase in the size and probably in the number of the ovarian hilus cells, just as it does in the testicular Leydig cells. The source of new hilus cells, however, deserves attention. Although occasional mitoses were observed in the cells of 2 patients treated with chorionic gonadotropin, we do not believe mitotic division of pre-existing hilus cells is the major mode of proliferation. Our observations suggest that the ovarian hilus cells differentiate from connective tissue cells indistinguishable from fibroblasts, in a manner similar to the differentiation of testicular Leydig cells (11, 12). Most of the hilus cells are congregated in the immediate vicinity of nonmyelinated nerves, and the sequence of differentiation of hilus cells is

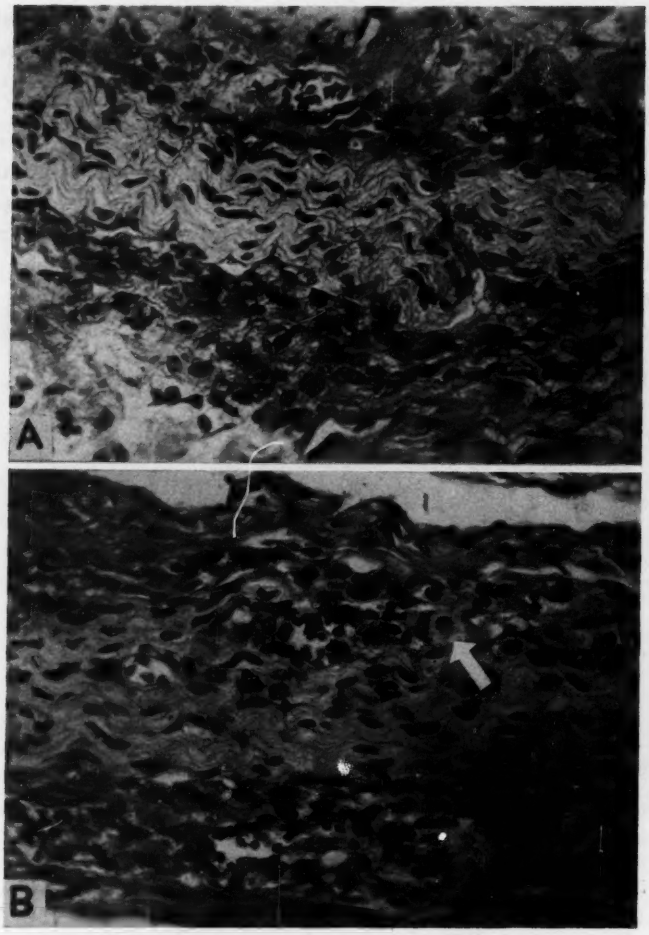

FIG. 11. Sequence in differentiation of ovarian hilus cells in 39-year-old patient who received chorionic gonadotropin, 10,000 I.U. daily for twenty-one days. 11A illustrates proliferation of a cuff of fibroblast-like cells about a nonmyelinated nerve. 11B shows an adjacent nerve with similar proliferation, but with hilus cells differentiating among the fibroblast-like cells. The hilus cells are polygonal, with more abundant, granular cytoplasm, sharper cell borders, rounded vesicular nuclei, and one or two nucleoli. ×270

best illustrated in relation to these nerves (Fig. 11). In ovaries stimulated by chorionic gonadotropin, conspicuous cuffs of plump fibroblast-like cells can be seen about the nerve trunks (Fig. 11A). These probably represent the first phase of differentiation from morphologically indifferent connective-tissue cells. At this stage the cells are fusiform with elongated nu-

clei, nongranular cytoplasm and indistinct cell borders; they have none of the characteristics of secreting cells. About adjacent nerve trunks or further along the same nerve, a further stage of differentiation may be seen. Among the fibroblast-like cells there appear occasional cells which are plumper and tend to be ovoid or polygonal rather than elongate, and in which the nuclei assume a spherical shape with one or more conspicuous nucleoli (Fig. 11B). Fine acidophilic granules have accumulated in the cytoplasm and the cell membrane has become more distinct. The cell now has the appearance of a secreting cell. In other areas about the nerve larger, well differentiated, polygonal hilus cells are present in abundance and few of the fibroblast-like precursors are seen. Occasional hilus cells also appear within the nerve bundle.

The close anatomic interrelationship of nerve and hilus cells is as puzzling as the identical association of nerves and extraglandular Leydig cells in the testis. One is tempted to speculate about neurohormonal mechanisms, but without more data such speculation would be premature. However, in studying these neural complexes under hormonal stimulation, one cannot help but feel that the nerve exerts some kind of "organizing" influence on the metamorphosis of hilus cells from indifferent connective tissue cells, since differentiation to secreting cells occurs predominantly in those connective tissue cells in close apposition to nonmyelinated nerve. Hilus cells are often found differentiating *within* the nerve trunk, between individual nerve fibers, but it is not possible to determine whether they arise from Schwann cells or from mesenchymal elements incorporated within the nerve trunk. The suggestion that nerves may exert an "inductor" influence in the differentiation of ovarian hilus cells is, of course, a matter of inference from the anatomic relationships. There are analogous situations, however, in which nerve elements exert an organizing influence on adjacent structures, as in the differentiation of taste buds from lingual epithelium. Taste buds, derived from lingual epithelium, degenerate on section of the nerve and again differentiate when nerves regenerate (15, 16). Unfortunately, ovarian hilus cells occur only in the primate species, so that experimental investigation of the relation of these cells to nerve is difficult.

17-Ketosteroid determinations. Determinations of urinary hormones (including glycogenic corticoids, prolactin, and 17-ketosteroids) on the group of patients treated with chorionic gonadotropin, have been recorded in a previous paper (10). Reference will be made here only to the urinary 17-ketosteroid determinations. Of the 6 women treated, 3 had a moderate sustained rise in 17-ketosteroids, as measured in each case by one determination before treatment and four determinations during treatment. The average rise in 17-ketosteroids was 6.2 mg. in the 24-hour urine specimen.

A fourth patient had a 24-hour increase from 5.1 mg. before treatment to 17.4 mg. during treatment, but in this case only a single determination was made during treatment. Two patients failed to show any rise in 17-ketosteroids during chorionic gonadotropin therapy. A spectacular rise was not anticipated, since it has been our experience that patients with masculinizing syndromes due to hilus-cell tumors or hyperplasia show either normal or only slightly elevated 17-ketosteroid excretion.

SUMMARY

1. Significant cytologic changes were observed in the ovarian hilus cells (Leydig-like cells) with administration of chorionic gonadotropin in women. The cells showed an increased size, acidophilic granulation of the cytoplasm and other changes suggestive of increased activity. Occasional mitotic figures and foci of degeneration were present.

2. Comparable changes were seen in ovaries of pregnant patients and in 1 instance of uterine chorioepithelioma. An increase in the number of hilus cells was particularly conspicuous in the term pregnancies. In general, the advance of pregnancy was reflected in an increase in numbers of hilus cells, increased size of cells and a greater degree of pleomorphism.

3. Four of 6 women treated with chorionic gonadotropin showed an increase in urinary 17-ketosteroids. Whether this is related to increased androgen production by ovarian hilus cells cannot be definitely established.

4. Ovarian hilus cells appear to arise by differentiation from connective tissue cell precursors, in similar fashion to testicular Leydig cells. The possibility is suggested that nonmyelinated nerve acts as an "organizer" in this differentiation.

5. The nature of the changes noted in response to chorionic gonadotropin furnish additional evidence that the ovarian hilus cells are functional, hormone-producing cells and that they are closely related to, or identical with, testicular Leydig cells.

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REMISSION IN CUSHING'S SYNDROME AFTER BILATERAL HEMIADRENALECTOMY*

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THE current interest in adrenal physiology encourages us to present a well documented case of Cushing's syndrome with certain instructive features. Whereas the diagnosis was made[1] when the patient was first observed, it was later obscured by an x-ray induced remission. The response of the patient to subtotal resection of the adrenals was prompt and spectacular in contrast to previous failure of sustained remission after pituitary irradiation. A very satisfactory remission has followed the operation despite the absence of histologic proof of hyperplasia or hypertrophy of the adrenals.

CASE REPORT

The patient's history is long and varied. To facilitate her case presentation, the physical findings and laboratory observations are recorded in chronologic order, together with the impressions of the different observers in New York, Newark, and Boston.

In May 1945,[1] when 13 years old, she was first studied and hospitalized with the chief complaint that, although she had always been somewhat short and dumpy, during the previous two years she had ceased growing altogether and had increased in weight rapidly, reaching 122 pounds in the summer of 1944. For the previous year she had dieted and her weight had fallen to 97 pounds, her height being 4 feet 8 inches. At this time there were no other complaints—no headaches or visual, urinary or other disturbances. The past history was essentially noncontributory, as was the family history. The menarche had not occurred. There was no history of abnormality of fat distribution, striae, plethora, hirsutism, or hypertension. Figure 1 shows the abnormalities in growth noted with the appearance of her disease. Her rate of growth prior to the age of 11 years had been well within normal limits.

Physical examination at this time showed a young, white female with a buffalo-type obesity, suprapubic fat pads and a ruddy complexion. Her height was 56 inches and her

Received for publication July 18, 1952.

* Read before the 33rd Annual Meeting of The Association for the Study of Internal Secretions, Atlantic City, N. J., June 7–9, 1951.

[1] The authors are indebted to Dr. Leo Davidoff for these early data.

weight was 103 pounds, giving her a very short and squat appearance. Her blood pressure was 156/98. Neurologic examination gave negative results.

X-ray examination showed the sella turcica to be rather small. The skull markings were normal, and there was no evidence of decreased mineralization.

The basal metabolic rate was −5 per cent. The concentration of blood calcium was 10.8 mg., sugar 94 mg., urea nitrogen 10.2 mg. and total cholesterol 227 mg. per 100 cc.

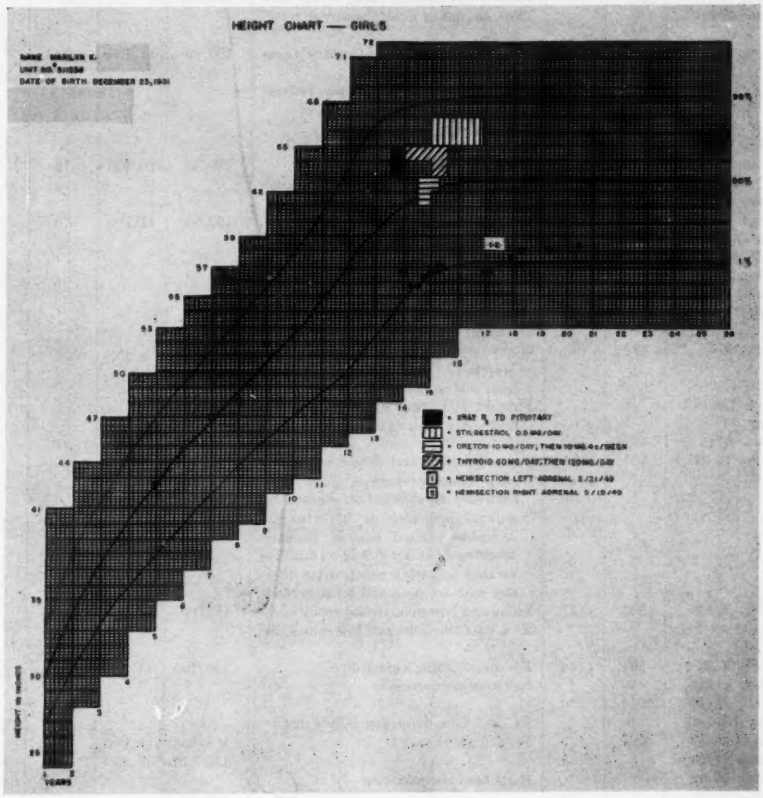

FIG. 1. Growth chart.

A diagnosis of pituitary basophilism was made and she was referred to a roentgenologist for radiotherapy. X-ray plates of the skull at that time were reported as showing evidence of an infantile sella. She received three courses of x-ray treatment to the pituitary. Twelve treatments were given in each course—four over each of three 5 cm. ×5 cm. fields centered over the sella, one anterior and two lateral. The dose was 200-r in air given at each treatment with the following factors: 200 KV., 10 ma., 50 cm. F.S.D., filter 1.3 Cu and 1.0 Al.

There was not much change in her condition six months after x-ray therapy, at which time (November 21, 1945) she was seen in *Boston*. Physical examination showed

TABLE 1. CLINICAL AND LABORATORY FINDINGS, IN CHRONOLOGIC SEQUENCE

Date	Ht. (in.)	Wt. (lbs.)	Appearance and therapy	Blood pressure (mm. Hg)	Blood count		
					WBC	Lymphs. %	Eos. (total)
May 1945	55	103	Buffalo-type obesity, ruddy complexion	156/98			
June–Sept. 1945			Three courses of x-ray therapy to pituitary				
Nov. 23/45	56	118	Somewhat suggestive of Cushing's syndrome	100/60	12,400	20	
Nov. 1945–Apr. 1946			Thyroid 60 mg. daily for 1 mo., 120 mg. daily for 4 mos.; low-calorie diet				
Apr. 24/46	55¼	106½	Unchanged				
May 6/46			No treatment				
July 8/46	55¼	114	Striae less marked	120/80	10,800	18	
July 8–Sept. 25/46			Thyroid 60 mg., and methyltestosterone 10 mg. daily				
Sept. 25/46	56¾	122½	Face rounder	132/94	14,700	29	
Sept. 25–Dec. 27/46			Thyroid 60 mg., and methyltestosterone 10 mg. daily				
Dec. 27/46	56¾	116	Slight acne				
Dec. 25/46–Apr. 9/47			Thyroid 100 mg. daily; methyltestosterone 10 Gm. 4 x wk. Stilbestrol 0.5 mg. daily				
Apr. 9/47	57¼	125	Slight acne. First menstrual period March 17, 1947, after patient had been taking stilbestrol for 3 mos.				
Apr. 9–Dec. 29/47			Thyroid 100 mg.; stilbestrol 0.5 mg. daily—for forty days on, then ten off				
Jan. 9/48	56¾	126		140/106	12,800	17	
June 15/48	56¾	126	Striae, plethora and dorsal hump. Insulin tolerance normal, June 1948. Perirenal air insufflation showed no adrenal neoplasm or hypertrophy. Atrophic vaginal mucosa (smear). Menstrual periods in August and November following substitution therapy with estrogen and progesterone	145/95			
Dec. 29/48	56¾	137	Striae and hump; increased ecchymoses	145/120	13,000	16	8
Dec. 29/48–Feb. 8/49			KCl, 6.0 Gm. daily, and low calorie diet				
Feb. 8/49	56¾	134	Definite Cushing's syndrome.	140/100	11,600	11	21
Feb. 21/49			*Left hemiadrenalectomy*				
Feb. 28/49	56¾	135					89
Mar. 1–20/49			KCl, 6.0 Gm. daily; low calorie diet				
Apr. 20/49	56¼	132	No definite change	128/108	9,000	25	50
May 17/49		135		130/110	13,200	24	
May 19/49			*Right hemiadrenalectomy*				
May 25/49		134		120/90			
May 25–July 15/49			Low calorie diet				
July 15/49			Face slightly thinner	120/95			176
Aug. 9/49			No treatment. Patient had two normal spontaneous menstrual periods. Vaginal smear showed secretory phase				
Nov. 16/49	57¼	109¾	Face still thinner	118/20	8,150	43	133
Apr. 5 1950	58	113	Entirely normal	115/70	8,500	40	98
Nov. 5/50	58¼	130½	Entirely normal, except for obesity. Regular and spontaneous menstrual periods each month.	110/75			
May 23/51	58¾	137	Ditto	128/80			108
May 27/52	58¾	148	Ditto	118/80			
June 3/52		150	Obese	110/80			

Blood chemistry					Urin. calcium mg./24 hrs.	Hormone assays (urine)		
						17-KS mg./24 hrs.	11-OCS* mg./24 hrs.	FSH mμ./24 hrs.
Na mEq./L	K mEq./L	Cl mEq./L	CO_2 mEq./L	Chol. mg. %				
				227				
140.8	5.5	96	30.4	305	84	2.7	0.33	3.0
140.7	4.6			278		4.4	1.02	6.5—13
						4.4	0.4	<6.5
						2.0	0.13	<6.5
				230				
	4.2	103	29.5	218		9.2	0.69	<6.5
137.0	4.8	94	32.2	190	234	8.6		
						5.1	.16	
	3.2	110	28.8		87	6.0 4.0		
						2.5 1.4	0.25	
						1.4 (5.2)** 1.0		
134.4						4.6**		
						2.0		
140.9						10.3**	1.3***	

* 11-Oxysteroid (normal range, 0.1–0.44 mg./24 hrs.).
** Performed in Newark (normal range, 7–17 mg. /24 hrs.).
*** Performed in New York (normal range, 0.6–1.8 mg./24 hrs.).

dryness of the hair on the head, beginning growth of axillary and pubic hair, obesity not confined to the abdomen and face, a "buffalo hump," a few pink striae on the abdomen such as one would expect with obesity, no acne, no pinkness of the skin, and no hypertension. Her appearance was suggestive, but not typical of, Cushing's syndrome (Fig. 2).

X-ray findings were essentially the same as those of May 1945. The bone age was 13 years and 3 months.

Laboratory findings at this and subsequent visits are recorded chronologically in Table 1. The results of a glucose tolerance test were as follows: fasting 91 mg., ½ hr. 105 mg., 1 hr. 120 mg., 2 hrs. 118 mg., 3 hrs. 103 mg., and 4 hrs. 96 mg. per 100 cc.

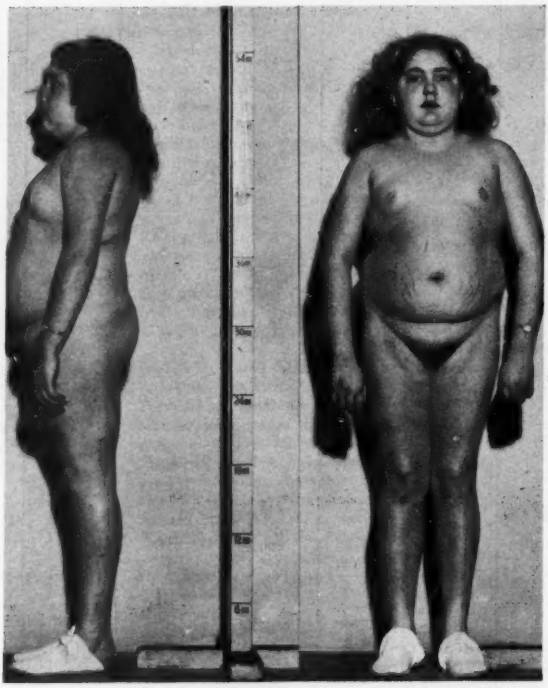

FIG. 2. Patient after x-radiation of the pituitary gland at 14 years of age.

It was concluded at this time that the patient did not have Cushing's syndrome. The serum potassium level was not low; the serum sodium level was not high; blood pressure was only 100/60; the lymphocytes were not depressed; the bones were not osteoporotic; and the tolerance for glucose was increased. That her 11-oxysteroid excretion was normal was considered, at that time, strong evidence against the diagnosis of Cushing's syndrome. Hypothyroidism was considered, in view of a high serum cholesterol concentration, an increased glucose tolerance, a failure to grow (Fig. 1) and low serum phosphorus and alkaline phosphatase levels. Thyroid therapy and a diet low in fat and starch were, therefore, recommended.

The patient was again seen in April 1946. At that time she had not grown, but her weight had dropped 11½ pounds. Her urine showed 2 plus albumin, many white cells and occasional red cells. There was no notable change in her physical appearance.

She returned in July 1946, having discontinued thyroid therapy in May. Her blood pressure was 120/80 and her striae had practically disappeared. The urine was normal. There was no other change in appearance. Other laboratory findings at this time are listed in Table 1. The significant changes were an increase of 11-oxysteroid excretion from 0.33 to 1.02 mg. per twenty-four hours (normal range, 0.1 to 0.44) and the appearance of a positive reaction for follicle-stimulating hormone $>6.5<13$ mouse units per twenty-four hours. She was given methyltestosterone, 10 mg. daily for six weeks, plus 60 mg. of thyroid daily.

In September 1946 her height was ½ inch less and her weight 8½ pounds more. She had not had a menstrual period. On physical examination some slight increase of hair on the arms and a mild acneiform eruption on the forehead were noted. There was no change in the voice. A catheter specimen of urine was entirely normal. Thyroid and methyltestosterone were continued at the same dose levels for three more months. Stilbestrol, in a dose of 0.5 mg. daily, was later added to the therapeutic regimen and the methyltestosterone was decreased to 10 mg. four times per week; the thyroid was increased to 100 mg. daily.

In April 1947 her height had increased ¾ inch and her weight 2½ pounds. She had had a period while taking stilbestrol. Physical examination showed the patient to be essentially unchanged. Acne was still present, she noted some increase of hirsutism on the face. Laboratory findings showed a decrease (probably resulting from methyltestosterone therapy) in 17-ketosteroid excretion to 2.0 mg. per twenty-four hours, and a decrease in 11-oxysteroids to 0.13 mg. per twenty-four hours. Methyltestosterone therapy was discontinued, but thyroid dosage was maintained and cyclic therapy with stilbestrol was instituted.

In December 1947 she was observed in *Newark*. She had menstruated in June and July following stilbestrol withdrawal. Height and weight were not significantly changed. The blood pressure had risen to 140/106. She had a generalized discreet erythematous papular rash on her face, chest and limbs and an enlarged clitoris. The rest of the examination gave essentially unchanged findings as did the x-ray examination. The eyes, including peripheral fields, were also normal. She was given a 1,200-calorie diet and *d*-amphetamine therapy.

When she was seen again six months later, in June 1948, there was no change in height, weight or blood pressure. She was obese and had marked plethora of the face. There was a mild acneiform eruption on the face and shoulders and moderate hyperkeratosis of the arms. There was an increase of the hirsutism on the upper lip, chin and malar surfaces. The skin was thin and showed evidence of excessive ecchymosis from recent trauma. Purplish striae were present on the abdomen. On examination the vagina was normal except for leukorrhea. The clitoris was within normal limits. The diagnosis of Cushing's syndrome was established and was based on the following observations: 1) obesity with moon face and buffalo hump, 2) plethora, 3) moderate hypertension, 4) abdominal striae, 5) a thin skin, easily bruised, 6) hirsutism, 7) acne, 8) hyperkeratosis, 9) amenorrhea, and 10) uncooperative attitude.

An insulin tolerance test (0.1 unit of regular insulin per kilogram of body weight administered intravenously) on June 18, 1948, showed the following normal curve: fasting 104 mg., 10 min. 74 mg., 20 min. 50 mg., 30 min. 65 mg., 45 min. 89 mg., 60 min. 122 mg., 90 min. 140 mg., and 120 min. 137 mg. per 100 cc.

Perirenal air insufflation[2] showed no evidence of adrenal neoplasm or hypertrophy. The vaginal smear suggested estrin lack.

The bone age was consistent with the chronologic age of 16.

The patient was next examined in *Boston*, December 29, 1948. It was agreed that her appearance was now that of Cushing's syndrome.

The laboratory findings tended to confirm this impression, in that the 17-ketosteroids and 11-oxysteroids had risen, whereas the assay for the follicle-stimulating hormone was

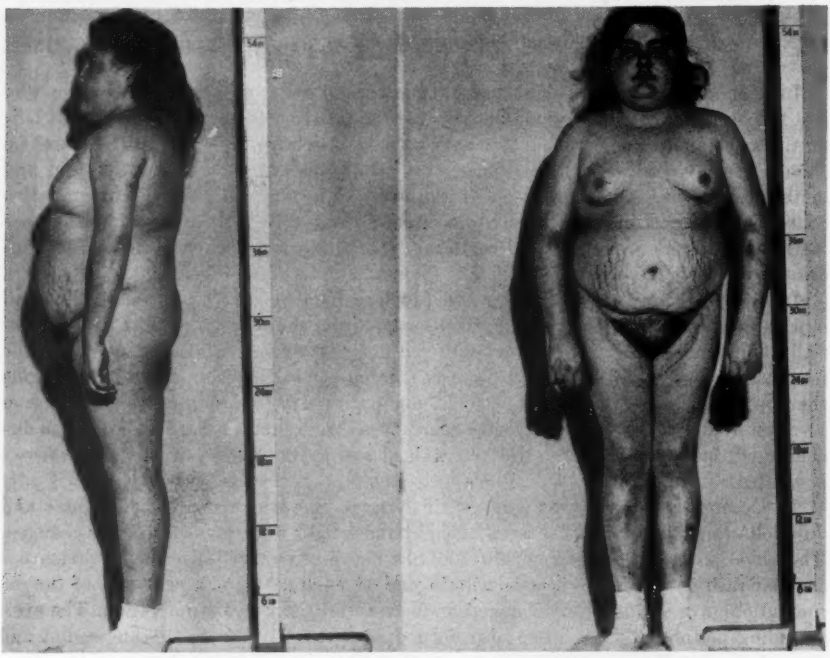

FIG. 3. Appearance of patient prior to initial adrenal surgery—18 years of age.

now negative for 6.5 units per twenty-four hours. The white blood cells had risen to 13,000, and the percentage of lymphocytes had fallen to 8.

X-ray examination of the thoracic and lumbar spine showed that the vertebrae were rather small in size and deficient in calcium.

The diagnosis of Cushing's syndrome was agreed upon and adrenal exploration was recommended. In the interim, she received a low calorie diet and potassium chloride.

On admission to the Massachusetts General Hospital, laboratory findings showed a decrease in the concentration of serum chloride and an increase in the serum carbon dioxide content. The urinary calcium had increased to *234 mg. per twenty-four hours* (see Table 1). The patient's appearance at this time is depicted in Figure 3.

The left adrenal was explored in February 1949. It appeared to be within normal limits of size and shape. Half of it was removed. Microscopically there was no proof of

[2] By Dr. George Cahill—Presbyterian Hospital, New York City.

hyperplasia or hypertrophy of the adrenal cortex. Eosinophil counts obtained before and after surgery were as follows:

	Cells per cu. mm.
Six days before operation	11
Five days before operation	38
Three days before operation	14
One day before operation	38
Day of Operation	0
First postoperative day	2
Second postoperative day	4
Third postoperative day	7
Fifth postoperative day	17
Seventh postoperative day	89
Eighth postoperative day	42

The postoperative course was exceedingly smooth, which precluded any evidence of adrenal insufficiency. The patient was discharged March 2, 1949, on a low calorie diet supplemented with *dl*-amphetamine sulfate and potassium chloride.

A check-up in April 1949 showed increased weight, with height unchanged. Blood pressure was 128/108. There was no notable change in appearance. The serum chloride level had increased to 110 mEq./L and the carbon dioxide content had decreased to 28.8 mEq./L. These changes were thought to be the result of potassium chloride medication.

It was decided to operate on the right adrenal. On May 19, 1949 half of the right adrenal was removed. There were no gross or microscopic changes consistent with the diagnosis of hypertrophy or hyperplasia of the adrenal cortex. The eosinophils, which were absent on the day of the operation, rose to 73 per cu. mm. on the sixth postoperative day.

On July 15, 1949 her weight was down to 123½ pounds; the blood pressure was 120/95. Laboratory data at this time showed that the eosinophils had risen to 176 per cu. mm. (normal); 17-ketosteroids had fallen to 1.4 mg. per twenty-four hours (low); and the 11-oxysteroids had fallen to 0.25 mg. per twenty-four hours (normal). Numerous observers were impressed with the change in the patient's appearance, particularly the narrower, less florid face.

On August 8, 1949 it was observed in *Newark* that she had lost 5 pounds and had gained ⅜ inch. The blood pressure was 120/85. There was less plethora of the face, and a decreased amount of hair on face, arms and legs. The interscapular fat pad had decreased. The abdominal striae were less prominent. Abdominal fat was still present, but it was flabby. Pubic hair was decreased in amount and the breasts had increased in size. A vaginal smear showed normal vaginal cell morphology.

The patient had her first spontaneous, normal menstrual period on July 26, 1949.

On August 25, 1949 her height was 56¾ inches. The Cushing-syndrome habitus had almost completely disappeared at this time.

On September 8, 1949 the urinary 17-ketosteroid level was 4.2 mg. per twenty-four hours.

On October 15, 1949 she showed further improvement. There was an additional gain in height and loss in weight. The blood pressure was down to 105/65, and the urinary

17-ketosteroids had risen to 5.2 mg. per twenty-four hours (November 2, 1949).

Improvement has since continued, so that she no longer exhibits clinical signs or laboratory evidence of Cushing's syndrome. By April 1950 her appearance was that of a normal 19-year-old girl (Fig. 4). The dry and fuzzy scalp hair had become soft and

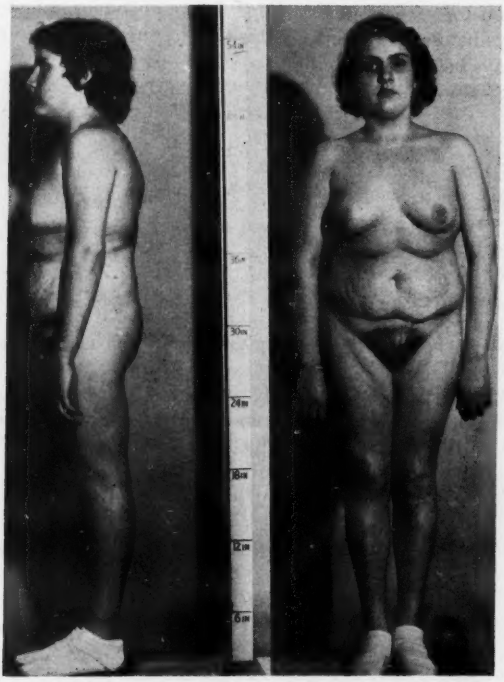

FIG. 4. One year after bilateral hemiadrenalectomy—19 years of age.

wavy, as well as lighter in color. The patient was last seen in June 1952, at which time her appearance was normal, except for obesity. There was no evidence of recurrence of the Cushing's syndrome. Normal laboratory findings prevailed. She has experienced normal, regular menstrual periods each month.

DISCUSSION

Now that classic Cushing's syndrome has been produced with cortisone, there is no longer any doubt that the disease is a form of hyperadrenocorticism. The fact that it can also be produced by ACTH shows that this hyperadrenocorticism, in turn, can be a manifestation of a hyperpituitarism with respect to ACTH. Heinbecker (1, 2) believes that this hyperpituitarism is, in turn, sometimes due to disorder of the hypothalamus.

It follows that treatment might theoretically be directed at any one of the following four levels: a) hypothalamus, b) pituitary, c) adrenal, and d) the tissues which constitute the end-organ. Three of these possibilities

have been explored with some success by means of radiation to the pituitary (3), hypophysectomy (4), subtotal adrenal resection (5, 6, 7), and the use of testosterone to counteract the tissue protein depletion which is one of the cardinal features of the syndrome (8–12).

In choosing from these forms of therapy, one would naturally like to advocate the least innocuous and most conservative first, and delay surgery until medical treatment has clearly failed. This can be justified only if operable adrenal cancer is ruled out. Adrenal tumor is the only cause of Cushing's syndrome recorded in infants and small children. The youngest patient with Cushing's syndrome due to adrenal hyperplasia which the authors have been able to find, was an 8-year-old girl. Although the authors believe that a low 17-ketosteroid excretion is highly suggestive of a benign adrenal tumor, they know of no finding which rules out malignant adrenal tumor in Cushing's syndrome.

Because of the possibility of the presence of an adrenal neoplasm, and because in severe cases the risk of surgery may increase rapidly with time, it is the authors' policy to advise exploration of one adrenal as soon as the diagnosis of Cushing's syndrome is clearly established. From the status of one adrenal the status of the other is computed and therapy planned.

The following rules summarize the authors' present view based on the study of 30 cases:

A. *If the first adrenal is large,* the patient has adrenal hyperplasia and the contralateral adrenal will be large also. Seventy per cent or more of the first gland is therefore removed at once. Surprisingly enough, this in itself may effect remission. If it does not, operation on the second side may be delayed while x-ray or hormone treatment is tried.

B. *If the first adrenal is abnormally small* (atrophied), the patient has a tumor in the contralateral adrenal and a second operation is clearly indicated. An assessment of the degree of atrophy in the first side may be helpful in predicting the severity of the adrenal insufficiency to be expected when the tumor is removed from the other side.

C. *If the adrenal is normal in size,* the patient has either 1) a tumor on the opposite side which has not yet caused contralateral atrophy (most unlikely), or 2) hyperfunction, which routine gross or microscopic examination of the gland does not reveal. In either case, subtotal resection is probably indicated, with more than 50 per cent of the adrenal tissue being removed at this time. Unless evidence of remission follows soon, the second adrenal should also be operated upon and resection accomplished.

SUMMARY

1. A case is presented of Cushing's syndrome developing in a girl of 11 years, observed from the age of 13 to her present age of 19.

2. There was a partial remission following x-ray treatment to the pi-

tuitary at the age of 13 years and a complete remission following bilateral subtotal adrenal resection at the age of 17 years.

3. The adrenals were not so definitely enlarged at operation that they could be identified as hyperplastic; they appeared normal on routine histologic examination. At operation, 50 per cent of the left and 60 per cent of the right adrenal were removed. Nevertheless, despite the gross and microscopic appearance of the two adrenals, any doubt that adrenal function was abnormal was dispelled by the marked clinical improvement noted after subtotal resection.

4. The patient has now been observed for two years postoperatively and has no signs or symptoms of Cushing's syndrome.

5. Growth, which had ceased abruptly with the onset of Cushing's syndrome, was resumed following subtotal adrenalectomy.

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VARIATIONS IN POTENCY OF ACTH PREPARATIONS: INADEQUACY OF PRESENT METHODS OF STANDARDIZATION*

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THE activity of the adrenal cortex in normal individuals is believed to be regulated by variations in the secretion of the pituitary adrenocorticotropic hormone (1–4). Administration of purified extracts of this hormone (ACTH) in amounts exceeding physiologic requirements, either as a protein substance or in the form of a peptide mixture (5), stimulates the adrenal cortex to greatly increased activity and with continued administration may induce a hyperplasia of the gland (7, 8). This action of the hormone is obviously dependent upon the presence of a responsive adrenal cortex, no effect being obtained in the absence of this gland. There has been some speculation as to whether there may be more than one adrenocorticotropic hormone (9, 10) but so far no conclusive evidence has been presented to warrant this assumption.

Commercial extracts of adrenocorticotropic hormone (ACTH) are readily soluble in aqueous solutions and are effective whether administered by intramuscular or by intravenous injection. It has been shown, however, that when ACTH is administered in a continuous intravenous drip over a period of six hours or longer, it is quantitatively more effective than when the same amount is given in repeated intramuscular injections (11–13). Some of the difference may be due to inactivation of the hormone at the site of intramuscular injection, but it is important to remember that a continuous intravenous infusion of a small quantity of ACTH provides a sustained stimulus over a prolonged period and resembles more closely the physiologic release of the hormone. Within certain limits, the effectiveness of the hormone by intravenous infusion appears to be more directly

Received for publication July 9, 1952.

* This study was assisted by a grant-in-aid from the Canadian Arthritis and Rheumatism Society.

The ACTH used was supplied in part by the National Research Council of Canada and the Department of Veterans' Affairs, Ottawa. Generous supplies were also received from the Armour Laboratories, Chicago, and the Connaught Medical Research Laboratories, University of Toronto.

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related to the duration of the infusion than to the actual size of the dose administered.

Since ACTH is not a pure substance, the hormonal activity of different batches may vary with each lot prepared. Commercial extracts have generally been assayed by the method of Sayers, Sayers and Woodbury (14) in which the ascorbic acid depletion of the adrenal cortical tissue of hypophysectomized rats is determined following the administration of measured amounts of the hormone. Earlier preparations were standardized in terms of milligrams of an original Armour standard (LA-1-A), but this was subsequently changed to International units and more recently to U.S.P. units, in an attempt to provide "some assurance of uniformity among the various brands of corticotropin" (15). However, the U.S.P. unit is essentially the same as the International unit and is defined as the specific biologic activity of 1 milligram of the original reference standard.

There appear to be wide variations both in the therapeutic and in the metabolic effects of ACTH as reported in the literature (5, 6, 9, 15–20). The differences have been variously attributed to a lack of responsiveness on the part of the patient concerned, an inherent resistance to the hormone, inactivation of the hormone at the site of injection, variations in the rates of absorption or the possible multiplicity of potent factors in the preparations used. The purpose of this report is to present evidence that much of the variability is due to differences in the potency of the hormone preparations; these differences have been much greater than is generally appreciated.

The observation that the labelled potency of ACTH may be in error and is not always reliable, has been made by a number of investigators (6, 15, 16). Equivalent doses of different lot numbers have often been observed to produce variable results in the same patient, and on occasions diseases under therapeutic control with one preparation have escaped from this control when another preparation was used. As in the case of many biologic products, the assay of ACTH is a difficult procedure, and in commercial manufacture it has been stated that a variation of less than 20 per cent in either direction may be regarded as good (6). It is a mistake to infer that an accuracy of this order is always obtained. Furthermore, if the assay is carried out before the material is dried, there is no assurance that the activity of the final product will be the same. Although the dried material appears to maintain its potency for long periods, it is possible that it may deteriorate slowly with time.

These variations in potency make it difficult to interpret much of the data related to the use of ACTH, particularly since the same lot-numbers have not always been used throughout clinical or metabolic studies, and since lot-numbers are frequently not stated in studies in which the antici-

pated results have not been obtained. It may not be of too great concern, providing that the variation is minor in degree; but recent reports have indicated that some preparations appear to be inactive when administered intramuscularly in recommended doses, yet are effective intravenously (13, 21). Our experience has shown that marked variations in potency of different lot-numbers of ACTH may be encountered, but we have yet to find a preparation which is ineffective intramuscularly, providing that sufficiently large doses are used. On one occasion for example, the dose had to be raised to as much as 800 milligrams per day intramuscularly, in order to obtain the desired effect.

Because the therapeutic requirement is much greater when the intramuscular route is employed (roughly, ten times the intravenous dose) differences in potency are magnified by the same factor when the intramuscular route is employed. These differences, however, can also be demonstrated when the preparations are given by the intravenous route, if sufficiently small doses are used. They are not apparent when the recommended intravenous dose of 20 milligrams is administered, since this is several times the amount required to produce a good therapeutic effect with a potent extract. It would seem that there is a narrow range of effectiveness of ACTH, with a critical upper limit beyond which no further adrenal stimulation can be produced. As a consequence, the use of excessive amounts does not appear to be either harmful or beneficial. On this basis, the selection of a dose of 20 milligrams of ACTH for slow intravenous administration has some merit in that, although the dose may be excessive, it avoids the possibility of using inadequate amounts of a preparation of low potency.

METHODS

The evaluation of the potency and effectiveness of different preparations of ACTH has been based primarily on the ability of the administered hormone to produce an increase in the excretion of 17-ketosteroids and corticoids in the urine, and its effectiveness in lowering the eosinophil count in the peripheral circulation in normal persons and in patients suffering from a variety of disorders.

The data have been correlated with the therapeutic effectiveness of the extracts in suppressing symptoms in patients suffering from rheumatoid arthritis, a disease which in our experience has shown the most consistent response to this hormone. It has also been supported by studies of the sedimentation rate and plasma fibrinogen in patients with rheumatoid arthritis, and in some instances by balance studies of protein and electrolyte metabolism. In addition to these observations, studies are under way to assess the extracts on the basis of their effectiveness in raising the serum corticoid levels.

Eosinophil counts have been made within five minutes of drawing blood, by the Dunger method (22, 23), using a 5 per cent eosin, acetone and formaldehyde mixture in distilled water. The urinary 17-ketosteroids have been estimated by a method essentially the same as the standard procedure of the Medical Research Council (24), and the corticoids by the method of Gornall and Macdonald (25).

RESULTS

The importance of using a single lot-number of ACTH throughout the period of a metabolic study was recognized early in the course of these investigations. Variations in the effectiveness of different preparations were observed, although many of these were not of sufficient magnitude to affect seriously the interpretation of results. However, over a period of two years discrepancies have been encountered which, on occasions, have been of so great a magnitude as to nullify the value of the metabolic data obtained. These findings have become a matter of some concern and hav- made it imperative to evaluate the potency of any preparation of ACTH selected for a study, prior to the commencement of the metabolic observations. This is particularly true, since most metabolic studies are conducted during intramuscular administration of the hormone. All of the data included in this report have been obtained with adrenocorticotropic hormone derived from hog pituitaries, except in one instance in which beef extract was used.

The results of one of our earlier studies in a patient with Reiter's syndrome are shown in Figure 1. This patient received 100 milligrams of ACTH daily for fourteen days, given in divided doses by intramuscular injection. A different lot-number was used for the last six days of the study. The data illustrate a minor difference in the effectiveness of the two preparations of hormone. As shown in the chart there was a good initial eosinophil suppression with lot H-4208, but this effect was lost within a period of six days. Evidence that the preparation was active is provided by the rise in the urinary 17-ketosteroids from a control value of 13.5 milligrams to a maximum of 32.1 milligrams per twenty-four hour period. However, with the change to a more active extract, lot J-10602, the eosinophils were immediately suppressed to zero values and remained there for the rest of the period. The urinary 17-ketosteroids also rose promptly to a value of 56.7 milligrams per twenty-four hours, almost double the value obtained with the first preparation. Studies of the sedimentation rate, plasma fibrinogen and serum proteins, uric acid excretion and nitrogen balance in this case did not reveal the difference in activity of the two extracts, possibly because they represent a less delicate measure or because the period of the study was too short.

A more obvious example of variations in potency is shown in Figure 2. ACTH, 100 milligrams daily, was administered by intramuscular injection in divided doses to a patient with rheumatoid arthritis. This amount of lot J-16508R was ineffective in suppressing the circulating eosinophils or in stimulating a rise in the urinary 17-ketosteroids, and had no beneficial effect on the symptoms of the disease. A change to lot C2-1 resulted in an immediate decrease in the eosinophil count and a rise in the urinary 17-ketosteroids from a value of 6.0 milligrams to 18.0 milligrams per twenty-

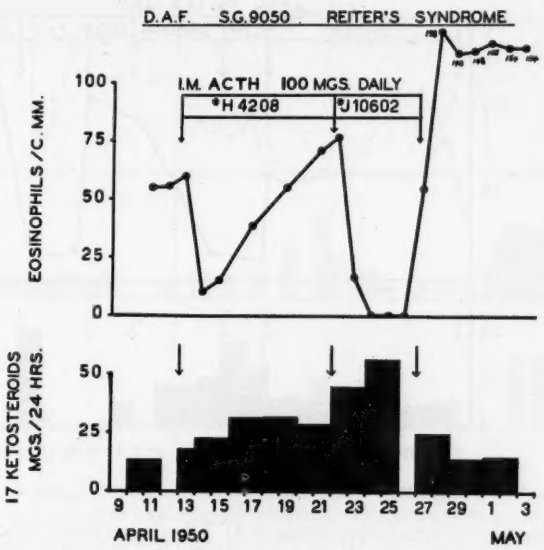

FIG. 1. Comparison of two preparations of ACTH given intramuscularly to a patient with Reiter's syndrome. Note that the eosinopenia was absolute and sustained with lot J-10602 and that the 17-ketosteroids reached values almost twice those obtained with H-4208.

four hours, during a four-day period. This extract produced good symptomatic improvement. When the first lot of ACTH (J-16508R) was resumed, the eosinophil count rose and the urinary 17-ketosteroids dropped to their control level. A trial with a third lot (J-22007) likewise had no effect but a fourth lot (C4-1) was effective clinically and again produced a prompt eosinophil suppression and an increase in the urinary 17-ketosteroids to 21.0 milligrams per twenty-four hours. The fact that a good response was obtained with two separate lot-numbers excludes the possibility of this difference being due to any abnormality on the part of the patient. Unfortunately no attempt was made in this instance to evaluate more ac-

curately the potency of the two ineffective extracts, although this has been done on subsequent occasions.

Figure 3 illustrates a part of a careful study in which an attempt was made to evaluate the relative activity of an extract of low potency as compared with one previously found to be effective. As is apparent from the chart, 100 milligrams of ACTH (lot J-19907R) administered by intra-

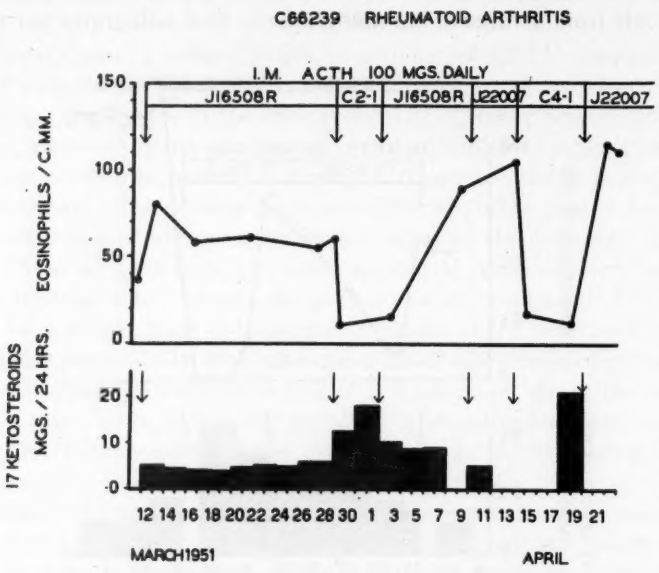

FIG. 2. Comparison of four preparations of ACTH administered intramuscularly to a patient with rheumatoid arthritis. Contrast the ineffectiveness of lot-numbers J-16508R and J-22007 in suppressing the eosinophil count and in increasing 17-ketosteroid production, as compared with lot-numbers C2-1 and C4-1.

muscular injection daily for eighteen days, resulted in only a transient suppression of the eosinophil count and no increase in the urinary 17-ketosteroids or corticoids. Symptomatically the patient was not improved. When the dose of hormone was raised finally to 400 milligrams per day, there was a distinct improvement in the patient's symptoms. With this amount the eosinophils were almost completely suppressed, while the 17-ketosteroids rose from 14.0 milligrams to 47.8 milligrams per twenty-four hours, and the urinary corticoids from 2.4 milligrams to 5.7 milligrams per twenty-four hours. When the original dose of 100 milligrams of ACTH was resumed, these figures returned approximately to their control values. Simultaneously there occurred a relapse in the symptoms of the disease. A change to a potent extract (lot C4-1) in a dose of 100 milligrams

daily, again resulted in a clinical remission. The eosinophils were immediately suppressed, while the urinary 17-ketosteroids rose to a value of 52.5 milligrams per twenty-four hours and the urinary corticoids to 8.8 milligrams. Confirmation of these results was provided by observations on the sedimentation rate and plasma fibrinogen (26), and metabolic data including nitrogen and electrolyte balance studies. A similar trial with these

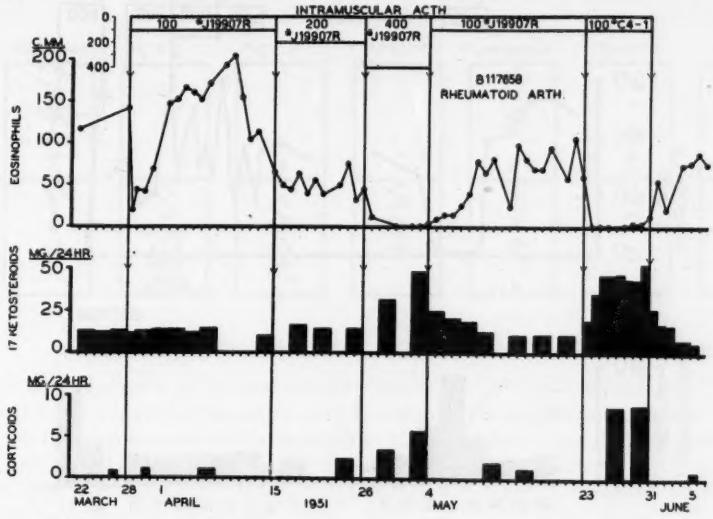

FIG. 3. Quantitative comparison of two preparations of ACTH administered intramuscularly to a patient with rheumatoid arthritis. The eosinopenia and the 17-ketosteroid increase produced by 100 milligrams daily of lot C4-1 were equal to those obtained with 400 milligrams of lot J-19907R.

two extracts in a second patient (an Rh-negative, pregnant woman) produced almost identical results.

The greatest discrepancy in the potency of ACTH encountered in this study is illustrated in Figure 4. In this instance daily doses of 100 milligrams, 200 milligrams and 400 milligrams of an experimental lot (C-KC-1) given intramuscularly for two-day periods to the same patient with rheumatoid arthritis, produced no clinical improvement and had no significant effect on the eosinophil count or 17-ketosteroids. Not until a dose of 800 milligrams daily was reached, was an effect produced which was comparable to that of 100 milligrams of lot C4-1.

In an attempt to evaluate these extracts by other means, a number of preparations have been tested for their effectiveness in suppressing the circulating eosinophils in response to the intramuscular injection of a single

dose of 25 milligrams of ACTH (27). This intramuscular test was originally proposed as a test for adrenal cortical insufficiency but has been subject to some criticism on the basis that it is not reliable and tends to give a high percentage of false positive results. For this reason it has been largely abandoned in favor of an intravenous test (28) which seems to give more consistent results. Actually the intramuscular test is a good one and can be

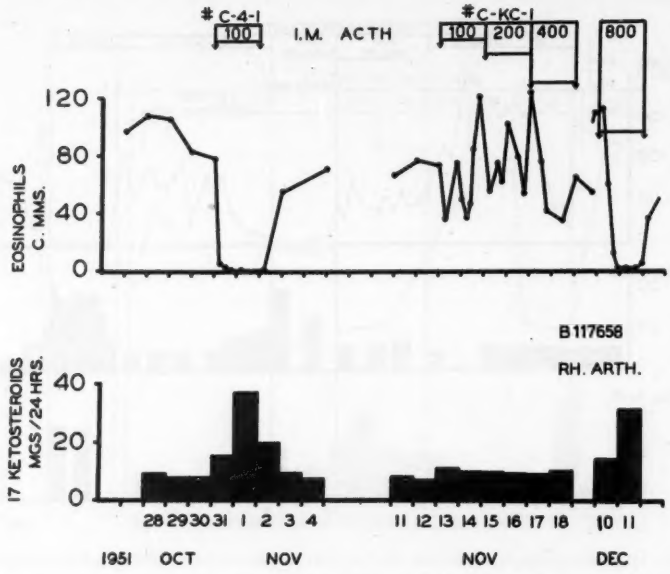

FIG. 4. Quantitative comparison of two preparations of ACTH administered intramuscularly to a patient with rheumatoid arthritis. The eosinopenia and the 17-ketosteroid increase produced by 100 milligrams daily of lot C4-1 were equal to those obtained with 800 milligrams of lot C-KC-1.

highly reliable, although it must be realized that it has certain limitations. Patients with Simmonds' disease, myxedema or schizophrenia often fail to give a normal response and must be excluded. Pernicious anemia, contrary to a recent report (19), has in our hands consistently given a normal response to this test, whether in remission or relapse, providing that a potent extract has been used. The interpretation of this test, as originally outlined, assumed that all batches of ACTH were of comparable potency. As has already been shown, this is not the case. Although there may be some inactivation of ACTH when it is given intramuscularly, in our experience this has not been an important factor in the variation between different lots. Providing that the extract is of known high potency, the test has been very reliable. With the intravenous test, a 20-milligram dose given by

slow infusion constitutes a much larger dose proportionately and hence will give more consistent results when ACTH of widely varying activity is used. Under similar circumstances the use of a larger test dose intramuscularly, such as 50 or 100 milligrams, will tend to give more uniform results.

An evaluation of some of the batches of ACTH previously mentioned, based on the eosinophil response to the intramuscular test, is shown in Figure 5. One must remember that there is a natural fluctuation in the

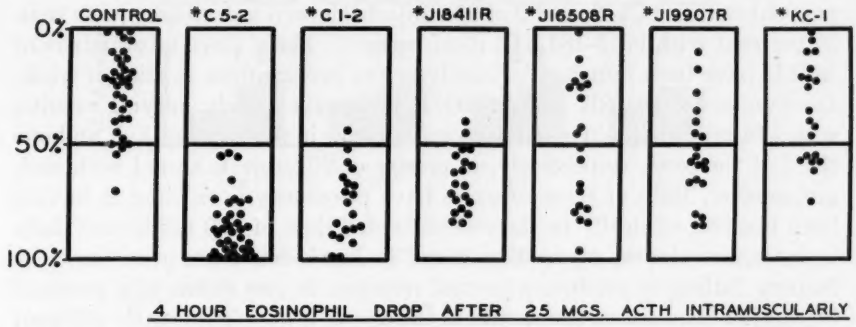

FIG. 5. Comparison of the eosinopenic effect of six different batches of ACTH, in response to the intramuscular injection of 25 milligrams of the hormone. The results are shown as the maximum percentile decrease at the end of four hours. Lot-numbers C5-2, C1-2 and J-18411R all gave highly consistent results. Lot-numbers J-16508R and J-19907R gave inconsistent results. Those obtained with lot KC-1 were not significantly different from those in the control group.

number of circulating eosinophils throughout the twenty-four hours of the day, sometimes associated with an appreciable decrease during the forenoon. With this in mind, eosinophil counts were made in a control group of 35 patients at hourly intervals, between the hours of 9:00 a.m. and 5:00 p.m. The maximum percentile drop for each patient in this group in any four-hour period has been plotted in the first column. It can be noted that a decrease in the count amounting to 50 per cent or more occurred in 5 instances, or in 14 per cent of the group. This finding is consistent with that reported by other observers (29–31). These 35 patients, who received no medication and were subjected to no stress-producing stimuli during the period of the test, serve as a control study for subsequent observations and may be taken to represent the average spread in the eosinophil count without stimulation. In the other columns is shown a comparison of the decrease in the eosinophils following the intramuscular injection of 25 milligrams of ACTH from six different batches, to groups of individuals consisting of 20 or more persons. As far as possible, each person was subjected to a comparison test with three or more different batches of ACTH, at in-

tervals of about seven days. All were shown to have a normal response with a known active extract. The tests were carried out between the hours of 10:00 a.m. and 4:00 p.m., meals not being excluded.

Six preparations of ACTH were studied in this manner. It is apparent that the extracts were not equally effective in suppressing the eosinophil count. All of the subjects showed a decrease of more than 50 per cent in 37 trials with lot C5-2. Only 1 patient, a case of myeloid metaplasia, out of a total group of 20 patients, failed to demonstrate a drop of more than 50 per cent with lot C1–2, and 2 of 20 subjects showed a decrease of less than 50 per cent with lot J-18411R, (beef extract). These three lot-numbers of ACTH have been found to be highly active preparations in clinical trials. Lot-numbers J-16508R and J-19907R both gave widely varying results, with a failure to obtain a satisfactory response in roughly one half and one third of the tests, respectively, in groups of 20 subjects tested with each preparation. Both of these extracts have previously been cited as having been inactive clinically in the recommended dose of 100 milligrams daily by intramuscular injection (Figs. 2 and 3). Lot C-KC-1 was even less satisfactory, failing to produce a normal response in two thirds of a group of 20 candidates. In fact, the spread in this group is not significantly different from that found in the control group. This was the extract previously cited as requiring 800 milligrams daily to produce a satisfactory clinical response by intramuscular injection (Fig. 4).

In order to exclude the possibility of inactivation of the hormone by intramuscular injection as a significant factor in this evaluation, an attempt was made to demonstrate similar variations in the activity of different extracts when the ACTH was administered intravenously. The difference in potency could not be demonstrated with intravenous administration when doses of 20 milligrams were given slowly over a period of six hours. However, when the dose was decreased progressively to a minimum of 1 milligram, a distinct difference in the effectiveness of the extracts was again apparent.

Figure 6*a* shows that, with lot C4-1, the intravenous infusion of 20, 10, 5 or even 1 milligram for a six-hour period on two successive days was almost equally effective in decreasing the eosinophil count. In our hands, extracts with a potency of this order have been used with consistently good therapeutic effect in doses of 5 milligrams daily, by a slow intravenous drip over six hours or longer. Amounts larger than this have not been more effective. With lot J-19907R, however (Fig. 6*b*) it can be seen that good effects were obtained with doses of 20, 10 and 5 milligrams, but there was a complete lack of eosinophil suppression with 1 milligram. Similar results have been observed on other occasions. During clinical trials in a patient with an undiagnosed disease of the collagen group (Fig. 7) a dose

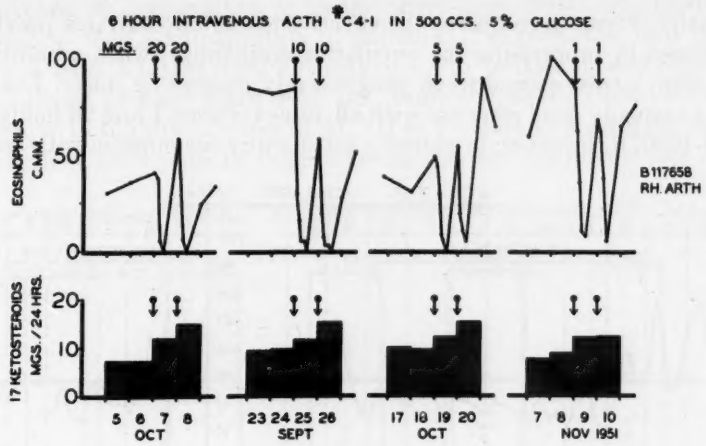

FIG. 6*a*

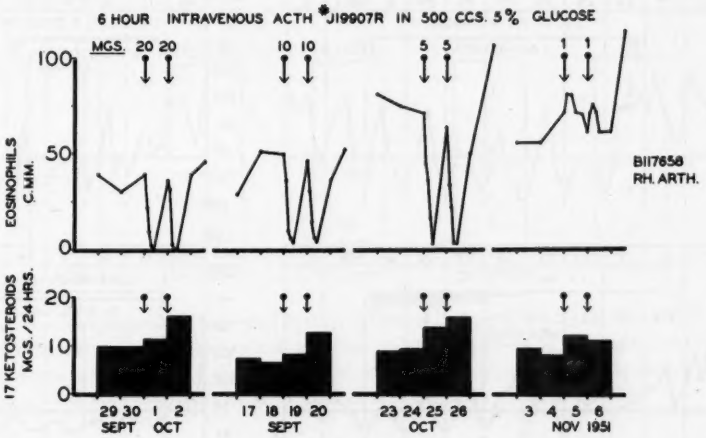

FIG. 6*b*

FIG. 6 (*a* and *b*). Comparison of two preparations of ACTH administered intravenously in varying doses over six-hour periods on two successive days with each dose. Lot C4-1 was almost equally effective in all doses from 20 milligrams to 1 milligram. Lot J-19907R was effective in doses of 20, 10, and 5 milligrams but was ineffective in suppressing the eosinophil count with a dose of 1 milligram.

of 0.5 milligram daily of lot C5-2 administered in this way, was more effective in controlling the febrile reaction and symptoms of the disease than either 1 or 2 milligrams of lot J-19907R. Extracts of the order of activity of the latter preparation have not always given satisfactory clinical results in doses less than 20 milligrams daily by slow intravenous infusion.

Finally, Figure 8 compares the effectiveness of two extracts previously mentioned in suppressing the circulating eosinophils, when administered as a slow intravenous drip in progressively decreasing doses. Lot C5-2 gave an equally good response with all doses between 1 and 20 milligrams. Lot J-19907R, however, produced a satisfactory eosinopenia with doses of

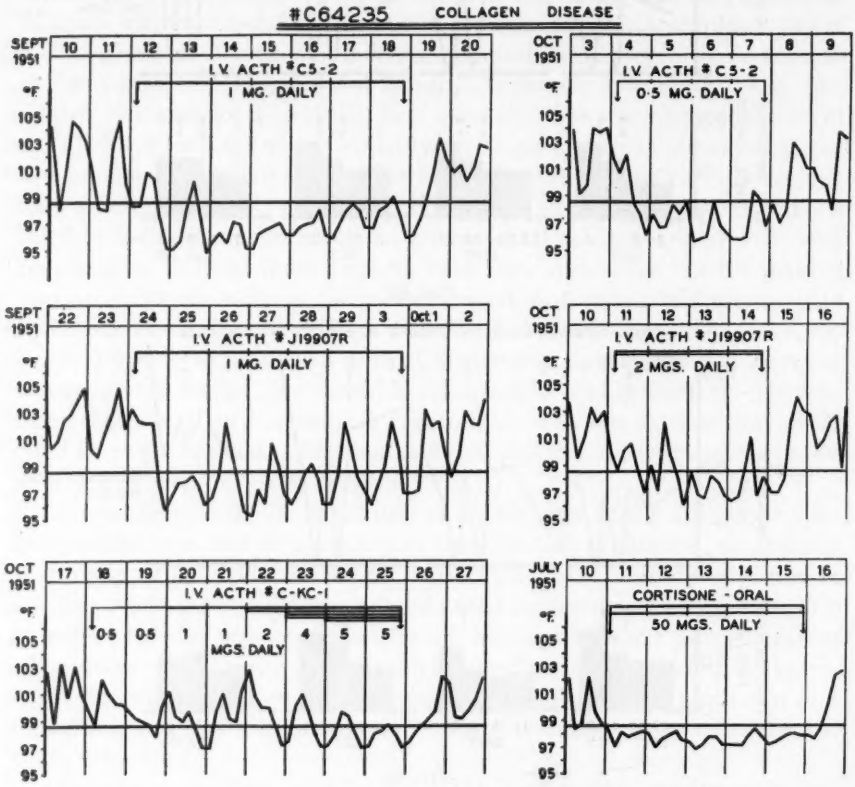

FIG. 7. Comparison of three preparations of ACTH administered intravenously over periods of six hours daily to a patient with an undiagnosed collagen disorder. Lot C5-2 in a dose of 0.5 milligram was more effective in suppressing the febrile response than 2 milligrams of J-19907R, and equal in effect to 5 milligrams of lot C-KC-1.

20, 10 and 5 milligrams, but failed in two instances out of ten trials with 2.5 milligrams and in six out of ten trials with 1 milligram.

DISCUSSION

The data presented clearly indicate that different batches of ACTH may vary widely in their biologic activity, whether measured by their clinical

effectiveness in disease states or by the physiologic effects they produce in response to their administration to patients. It is presumed that the extracts all gave a satisfactory bioassay prior to packaging and that they were labelled in accordance with the results obtained. Observations on differences in the effectiveness of ACTH as measured by a variety of procedures have been made by other investigators but the importance of this

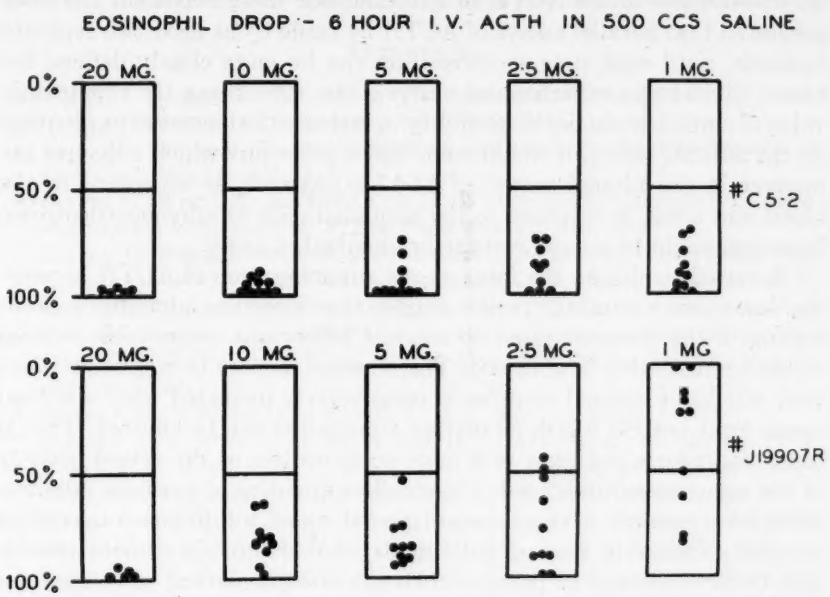

FIG. 8. Comparison of the eosinopenic effect of two preparations of ACTH administered intravenously over periods of six hours in varying doses. The results are expressed as the maximum percentile decrease at the end of eight hours. The eosinopenic effect of 1 milligram of lot C5-2 was equal to that of 5 milligrams of lot J-19907R.

finding has not been sufficiently stressed. It must of necessity be taken into account in any clinical or metabolic evaluation in which the expected changes do not occur or in which they differ markedly from well established actions of the hormone. To attribute these differences in activity to variations in the responsiveness of the individual patient, hormone resistance (32), tissue inactivation of the hormone, or the possible existence of a multiplicity of adrenocorticotropic hormones with different actions, even though it is realized that these factors may play a part, is not warranted until the extract in question has been retested for potency.

The activity of adrenocorticotropic hormone can be determined by a variety of different methods, but there appears to be no close parallelism

in the responses of any two test methods to the action of any two preparations (9). Although the ascorbic acid depletion test has been useful in measuring the adrenal cortical response to ACTH administered to hypophysectomized rats, it does not overcome differences that are known to exist between animal and human responsiveness. It cannot always be correlated with the secretory activity of the adrenal cortex or with the therapeutic effectiveness of the ACTH in question. For these reasons it has been suggested that parallel assays of ACTH be made by at least two separate methods, until such time as correlation can be more clearly defined between clinical and experimental observations (20). Since the therapeutic value of corticotropin lies in its ability to increase the output of oxysteroids by the adrenal cortex, it would seem that a procedure which measures the increase in the adrenal output of 11-17-oxysteroids, as estimated in the blood and urine, in response to the administration of adrenocorticotropic hormone, might be a more satisfactory method of assay.

Observations during the intravenous administration of ACTH in varying doses over a constant period suggest that a certain minimum concentration of the hormone must be reached before any appreciable adrenal cortical stimulation is obtained. There seems then to be a narrow range over which the adrenal stimulus is progressively increased, with a critical upper limit beyond which no further stimulation can be effected. This, if true, might form the basis of a more accurate test of the actual potency of the hormone administered. The smallest quantity of hormone administered intravenously over a constant period which will induce a maximum adrenal response in normal individuals, should provide a more reliable unit of measurement of potency than the present method of comparison to an arbitrarily selected standard (LA-1-A). Studies are under way to test the validity of these observations, using some of the extracts mentioned previously.

SUMMARY

Different batches of adrenocorticotropic hormone (ACTH), standardized on the basis of animal assay, may show considerable variations in potency when measured by therapeutic effectiveness, eosinopenic response, and ability to stimulate the adrenal cortex in patients with rheumatoid arthritis. This variation in potency between different extracts is apparent, whether the hormone is administered by intramuscular or by intravenous injection, and emphasizes the need for a more accurate standardization of the product. No extracts have been found to be totally lacking in therapeutic effect or in their ability to stimulate adrenal cortical function. With some preparations, however, the dose required to produce the same clinical and metabolic effect may be as much as four to eight times

that of others. The superiority of human assay of ACTH, particularly with respect to therapeutic effectiveness, is suggested.

Acknowledgment

Grateful acknowledgment is made to Mrs. M. Pamela Macdonald and Miss D. Strachan for technical assistance in the steroid assays, and to Miss S. Fraser, Miss K. Hollis and Miss H. German for technical assistance in performing the eosinophil counts.

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SOME EFFECTS OF ADRENOCORTICOTROPIC HORMONE IN VARIOUS HYPO-OVARIAN STATES. II. PRIMARY AMENORRHEA, SURGICAL CASTRATION, AND THE MENOPAUSE*

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IN a previous communication (1) we described certain effects of ACTH administered to 13 women with secondary amenorrhea. The present report deals with 8 other patients studied at the same time—2 with primary amenorrhea, 2 who were in the menopause, and 4 who had been surgically castrated. The purpose of the experiments was to determine whether complete absence of ovarian function would alter the metabolic and clinical effects of ACTH, as compared with its effects in women with partial ovarian failure. The methods of study were identical with those described for the first 13 cases. Table 1 gives the scheme of numbering of the patients, as well as certain essential clinical data, for the 8 subjects of this study.

Detailed protocols for 5 of the 8 patients follow:

Case 14. Mrs. D. E. was a 33-year-old white married woman. She had never had a spontaneous normal menstrual period. Pubic and axillary hair appeared at the age of 14–15 years, and the breasts began to develop at about the same time. Treatment with estrogens was begun when she was about 19 years old, and uterine bleeding finally occurred when she was 21 years old. Thereafter she was treated with various estrogens, anterior pituitary substance, and thyroid substance. Uterine bleeding occurred periodically only when estrogens were taken regularly. Estrogens were stopped on July 1, 1950, and slight vaginal bleeding occurred on August 10, 1950. There was no history of sexual abnormality or abnormal menstruation in the family. The patient was 170 cm. tall and weighed 62.3 Kg. The results of physical examination were entirely normal. Pubic and axillary hair were normal in character and amount; the breasts were normal. Pelvic examination gave normal findings, except that the uterus was small. X-ray examination showed the sella turcica to be normal. The basal metabolic rate was −6 per cent (Mayo Foundation standards). Routine laboratory examination of the blood and urine gave normal results. The urinary gonadotropin excretion was not determined. The patient was treated for eight days with ACTH (Armour) given intramuscularly at six-hour intervals in doses of 100 mg. daily. Since she had been taking an empiric dose of thyroid

Received for publication July 15, 1952.

* This work has been carried out under a grant from the Douglas Smith Foundation for Medical Research.

substance (0.130 Gm. daily) for several years, this was continued throughout the treatment period. No uterine bleeding occurred up to April 12, 1951, when the patient was told to resume taking stilbestrol 0.5 mg. daily.

The data shown in Figure 1 were obtained during the ACTH treatment period—October 3 to October 10, 1950, inclusive. The patient received a constant diet of C 192,

TABLE 1. CLINICAL DATA PERTAINING TO ADMINISTRATION OF ACTH IN HYPO-OVARIAN STATES

Patient (No. & initials)	Age (yrs.)	Diagnosis	No. of days ACTH admin'd.	Total amount of ACTH (Gm.)	Av. daily dose (mg.)	Uterine bleeding after treatment	Pretreatment urinary gonadotropin
14 (D.E.)	33	Primary amenorrhea; etiology unknown	8	0.800	100	None	Not assayed
15 (S.B.)	33	Primary amenorrhea; etiology unknown	8	0.800	100	None	High
16 (M.G.)	40	Menopause	7	0.700	100	None	Normal
17 (L.S.)	54	Menopause	8	0.800	100	None	High
18 (B.A.)	30	Bilateral salpingo-oophorectomy; total hysterectomy	8	0.800	100	None	Not assayed
19 (A.C.)	36	Bilateral salpingo-oophorectomy:					
		Preoperative	10	1.000	100	None	Normal
		Postoperative	8	0.800	100	None	High
20 (E.S.)	33	Bilateral oophorectomy	10	0.975	97.5	Slight	High
21 (D.W.)	30	Bilateral salpingo-oophorectomy; incomplete hysterectomy	8	0.800	100	None	High

P 84, F 78, calories 1,806, containing an estimated 13.45 Gm. of N, 1.477 Gm. of Na, 2.550 Gm. of Cl and 2.891 Gm. of K. She received in addition 3 Gm. of NaCl, added at the table. Distilled drinking water was allowed without restriction; the daily fluid intake varied from 660 to 1,410 cc.

Case 15. S. B. was a 33-year-old married white woman who had been followed in the out-patient clinic for fifteen years because of primary amenorrhea. She had never had any spontaneous uterine bleeding. There was no family history of menstrual or endocrine abnormality. At the time she was first seen, in 1936, she was 168 cm. tall and weighed 62.4 Kg. The breasts were preadolescent in size and configuration. Pubic and axillary hair were normal. The external genitalia were hypoplastic, the vaginal epithelium was infantile in character, and the uterus was quite small. The left ovary could be felt, but it was small; the right could not be felt. Visual fields were normal. X-ray examination showed the sella turcica to be normal. X-ray examination of the right hand showed osseous development consistent with a bone age of 13½ years, by Todd's standards. The basal metabolic rate was −16 per cent. Routine laboratory examination of the blood and urine gave normal results. Subsequently, treatment with various estrogens

produced considerable development of the breasts, increased growth of the pubic hair and cyclic, artificial uterine bleeding. At the time of the present study she was 173 cm. tall and weighed 64.4 Kg. Therapy with stilbestrol was stopped two and a half weeks prior to admission. Urinary gonadotropins, determined at the time of admission on April 9, 1951, were elevated to menopausal levels, *i.e.*, greater than 16 rat units per twenty-four hours (normal = 6–8 rat units per twenty-four hours). The patient was treated for eight days (April 17 through 24, 1951) with ACTH (Armour) given intra-

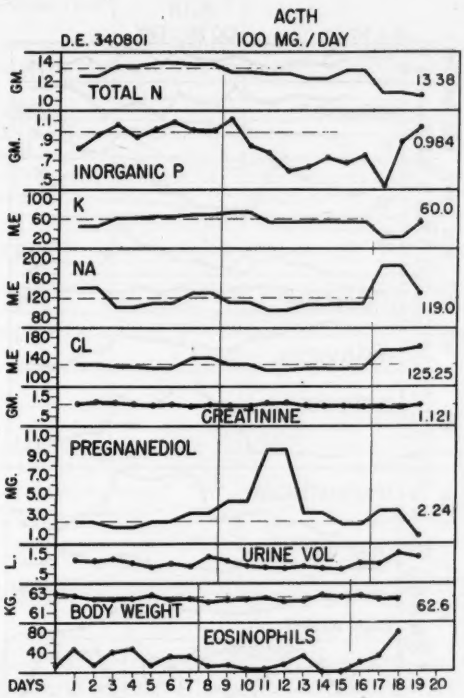

FIG. 1. The effects of ACTH on several urinary constituents and on total circulating eosinophils and body weight of D. E. (Case 14). The horizontal interrupted lines and the corresponding figures to the right mark pretreatment baselines. Isolated dots mark individual daily determinations; otherwise, two-day pools provide the information expressed by running lines.

muscularly at six-hour intervals, in doses of 100 mg. daily. Subsequent uterine bleeding had not occurred up to May 31, 1951.

Case 16. M. G. was a 40-year-old married white woman, para 0, gravida 0, who had not menstruated for five months at the time she was first seen in January 1951. Although she was not having hot flushes, the clinical impression was menopausal amenorrhea. The patient was 160 cm. tall and weighed 85.9 Kg. The results of physical examination, including the pelvis, were normal except for moderate obesity. Routine laboratory

examination of the blood and urine gave normal findings. Urinary gonadotropins were within the normal range. The basal metabolic rate was +7 per cent at the time of admission and −16 per cent at the time of discharge. The patient had been taking thyroid substance (0.24 Gm. daily) prior to admission; it was continued in a dose of 0.18 Gm. daily throughout the period of study.

The patient was treated for seven days with ACTH (Armour) given intramuscularly at six-hour intervals in doses of 100 mg. daily, from February 7 through 13, 1951. Uterine

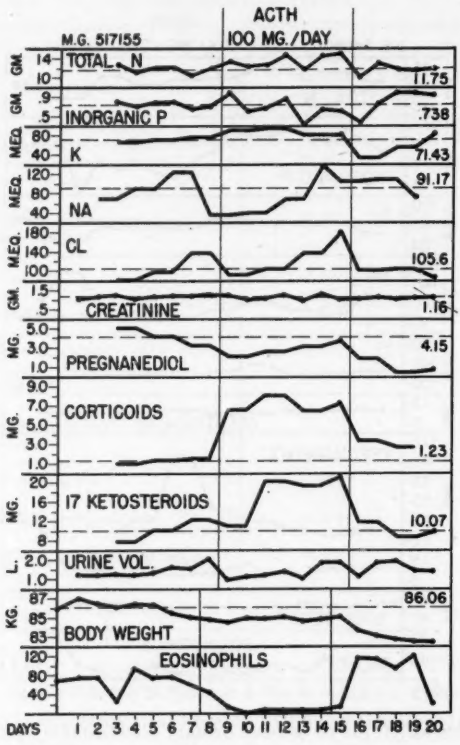

FIG. 2. The effects of ACTH on several urinary constituents and on total circulating eosinophils and body weight of M. G. (Case 16). For key, *see* legend to Figure 1.

bleeding occurred on February 10, 1951, according to the patient, but this was not confirmed by any of the nursing or medical staff. No subsequent uterine bleeding occurred. The data shown in Figure 2 were obtained during treatment with ACTH. The patient received a constant diet of C 83, P 67, F 21, calories 789, containing an estimated 10.72 Gm. of N, 0.814 Gm. of Na, 1.568 Gm. of Cl and 2.364 Gm. of K. She received in addition, 3 Gm. of NaCl added at the table. From February 6 through 14, 1951, she was given 2.7 Gm. of KCl daily in three divided doses.

Case 17. L. S., was a 54-year-old married white woman in the menopause, whose last menstrual period occurred in March, 1948. Hot flushes had been controlled by stil-

bestrol, but this was stopped on March 23, 1951. She continued to have periodic, severe headaches which were thought by several psychiatric and neurologic consultants to be functional in nature. She was the mother of one healthy normal child and had had one spontaneous abortion. The past medical history was otherwise noncontributory. The patient was 161 cm. tall and weighed 61.0 Kg. Physical examination, including the pelvis, gave normal findings. The results of routine laboratory examination of the blood and urine were normal. The basal metabolic rate was −10 per cent. Urinary gonadotropins were assayed in a 24-hour specimen collected March 29–30, 1951, and were found to be high—in the menopausal range.

Case 19. A. C. was a 36-year-old married white woman admitted for hysterectomy and castration because of excessive and irregular uterine bleeding. She had had four

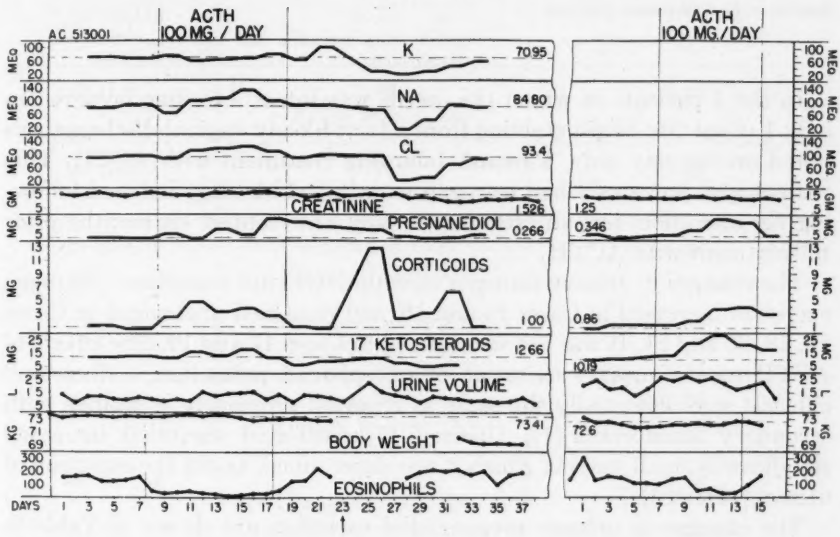

FIG. 3. The effects of ACTH on several urinary constituents and on total circulating eosinophils and body weight of A. C. (Case 19). The vertical arrow below the abscissa indicates the day of operation. For key, *see* legend to Figure 1.

pregnancies which resulted in two living children. The other two died within a few hours of birth. The patient was 172 cm. tall and weighed 73.4 Kg. The findings on physical examination, including the pelvis, were normal except for some thickening of the left adnexal region. Routine laboratory examination of the blood and urine gave normal results. The basal metabolic rate was within normal limits (−16 per cent, Mayo Foundation standards). The patient was treated for ten days with ACTH (Armour) given intramuscularly at six-hour intervals in doses of 100 mg. daily from December 26, 1950 through January 4, 1951. On January 10, 1951, a complete hysterectomy and a bilateral salpingo-oophorectomy were performed. The ovaries were found to be normal by gross and microscopic examination. The patient made an uneventful recovery and was discharged from the hospital. She reported that hot flushes developed for the first time about February 18, 1951. No estrogenic therapy was prescribed, and the patient

re-entered the hospital in April 1951 for a second period of study. ACTH (Armour) was administered exactly as before in doses of 100 mg. daily for eight days—from April 8 through 15, 1951. The hot flushes were not affected by ACTH treatment.

Urinary gonadotropin excretion was determined December 20, 1950 and on January 23, 1951. It was within normal limits. On April 7, 1951, the gonadotropin excretion had increased to within the menopausal range. The patient received a regular diet during both periods of study. Ordinary table salt was unrestricted, and a supplement of 2.7 Gm. of KCl was given in three daily divided doses from April 8 through 16, 1951. During both periods, distilled drinking water was allowed without restriction. The oral fluid intake during the first period ranged from 530 to 2,280 cc.; during the second period it was not measured.

In Figure 3 is shown graphically the urinary excretion of certain metabolic products during both treatment periods.

RESULTS

In the 5 patients in whom the uterus was intact, bleeding occurred in only 1 (Case 20). Slight staining from a faint bloody vaginal discharge was noted on one day only, a month following treatment with ACTH. This patient had previously had estrogen-withdrawal bleeding induced following her operation, but all estrogen therapy was stopped six months prior to treatment with ACTH.

The changes in urinary nitrogen excretion were not consistent. Nitrogen excretion increased in Cases 15 and 16, and remained unchanged in Cases 14, 18, 20 and 21. It was not determined in Cases 17 and 19. The effects of ACTH on the urinary excretion of phosphorus, potassium, sodium and chloride were essentially the same as reported previously in women with secondary amenorrhea (1). Urinary 17-ketosteroid excretion increased significantly in all cases in which it was determined, as did the excretion of urinary corticoids.

The changes in urinary pregnanediol excretion are shown in Table 2. It will be seen that in 4 of the 8 patients ACTH caused a significant increase in pregnanediol excretion (Cases 19 and 20 are excluded because the increase was so small that it lay beyond the limit of sensitivity of the method). In 3 of these 4 patients (Cases 14, 15 and 17) the ovaries were intact anatomically; in the fourth patient (Case 21) the ovaries were absent. The fact that ovariectomy and total hysterectomy had no influence on the response of urinary pregnanediol to ACTH in Case 19 is suggestive, but needs confirmation to be accepted as conclusive evidence. Nevertheless, if a conclusion is to be drawn from this evidence, it must be that the ovarian contribution to the increase in urinary pregnanediol produced by ACTH is negligible, and that in all probability the adrenal cortex is the source.

TABLE 2. CHANGES IN URINARY PREGNANEDIOL DURING ACTH THERAPY

Patient (No. & initials)	Mg. pregnanediol per 24 hrs.	
	Average before ACTH	Maximum during ACTH
14 (D.E.)	2.24 (Range, 1.61–3.07)	9.50
15 (S.B.)	0.68 (Range, 0.48–0.84)	3.32
16 (M.G.)	4.15 (Range, 3.21–5.06)	3.74
17 (L.S.)	0.48 (Range, 0.36–0.60)	3.59
18 (B.A.)	0.76 (Range, 0.70–0.84)	0.85
19 (A.C.)	Preoperative, 0.27 (Range, 0.26–0.27)	1.69
	Postoperative, 0.35 (Range, 0.22–0.47)	1.65
20 (E.S.)	0.7 (Range, 0.55–0.92)	2.16
21 (D.W.)	0.98 (Range, 0.38–1.43)	3.32

SUMMARY

1. ACTH was administered for periods of from seven to ten days to 8 women with presumably complete absence of ovarian function.

2. Uterine bleeding subsequent to this treatment occurred in only 1 patient.

3. The metabolic effects of ACTH in these cases did not differ from those previously reported.

4. The presence or absence of the ovaries did not appear to influence the effect of ACTH on urinary pregnanediol excretion.

Acknowledgments

The authors are indebted to Dr. Richard Landau and Miss Ruth Loughead for expert technical assistance; to Mrs. Regina Malloy and Mrs. W. Stateson of the nursing staff of

the Chicago Lying-in Hospital, and to the members of the house staff of the Chicago Lying-in Hospital who assisted in this study.

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METABOLIC AND CLINICAL EFFECTS OF METHYLANDROSTENEDIOL IN HUMAN SUBJECTS*

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SINCE Kenyon and his associates first demonstrated the growth-promoting effects of testosterone compounds (1), considerable effort has been devoted to the search for a steroid compound which would have the anabolic properties of testosterone, without its androgenic effects. Among the compounds so studied is methylandrostenediol (17α-methyl-Δ^5-androstene-3β-17β-diol).

Methylandrostenediol was first synthesized by Ruzicka, Goldberg, and Rosenberg (2) in 1935. Tschopp (3) and Ruzicka and Rosenberg (4) in 1936 demonstrated androgenic activity when large amounts of the compound were administered to experimental animals. In capons, Deanesly and Parkes (5) found its androgenic potency to be one tenth that of testosterone and one half that of methyltestosterone. In mice, Homburger, Forbes and Desjardins (6) reported that methylandrostenediol produced insignificant androgenic effects. In rats, Gordan, Eisenberg and Moon (7) found no androgenic effects in doses of 1 milligram per kilogram but did find effects in doses of 50 milligrams per kilogram, and Deanesly and Parkes (5) felt that methylandrostenediol was one half to two thirds as androgenic as testosterone or methyltestosterone.

Anabolic properties of methylandrostenediol in animals have been described by Gordan, Eisenberg and Moon (7), and its renotropic effects have been pointed out by Homburger, Forbes and Desjardins (6), and by Kochakian (8, 9). A progesterone-like action of methylandrostenediol has been demonstrated in rabbits by Klein and Parkes (10) and in hypophysectomized rats by Masson (11). Masson also showed that methylandro-

Received for publication June 17, 1952.

* These studies have been supported in part by a grant from the Schering Corporation. Grateful acknowledgment is made to Dr. Edward Henderson, Research Director, Schering Corporation, for generous supplies of methylandrostenediol and testosterone propionate.

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‡ Schering Clinical Research Fellow, 1948–1949. Damon Runyon Clinical Research Fellow, 1949–1951.

stenediol had a spermatogenic activity comparable to that of Δ^5-pregnenolone.

In mice fed methylandrostenediol, retardation of tumor growth comparable to that obtained with methyltestosterone has been described by Foley (12). In 3 of 7 women with metastatic carcinoma of the breast, objective regression of soft tissue and bone lesions (similar to the effect of testosterone propionate) has been noted by Homburger, Kasdon and Fishman (13).

In man, Gordan and associates (14) have stated (without reporting data) that weight gain and nitrogen retention occur, and that there is abolition of pain and hypercalciuria in patients with osteoporosis and osteitis deformans following doses of 20 to 40 mg. per day of methylandrostenediol sublingually. Other investigators (13, 15–17) with methylandrostenediol have noted from one third to one half the nitrogen retention seen with comparable doses of testosterone, methyltestosterone, or testosterone propionate. None of these investigators, however, has demonstrated significant weight gains in any of their patients, when methylandrostenediol was given over either long or short periods of time in doses ranging from 20 to 200 mg. per day. Thorn (16), Wilkins (18, 19) and their associates have suggested that the nitrogen retention seen during methylandrostenediol therapy is so small as to be within the error of the method.

Virilization has not been reported with the use of methylandrostenediol in doses of 200 mg. per day or a total of 28 Gm. over a period of months. Talbot *et al.* (15) have described some mammary hyperplasia in an 8-year-old female Addisonian who received 50 mg. of methylandrostenediol by mouth for eighteen days. No breast stimulation occurred in the same patient during administration of any of the testosterones.

Henderson and Weinberg in an excellent review of the subject (20) have pointed out that, in the usual dose range, "toxicity of the compound is not a clinical consideration."

The present report includes relatively long-term metabolic studies in 3 patients.

METABOLIC STUDIES

Three young female patients were followed under long-term balance conditions on the metabolic ward:

1. Patient SHE, a 20-year-old female dwarf with panhypopituitarism.
2. Patient MWIL, a 28-year-old woman with "idiopathic" hirsutism.
3. Patient BAL, a 28-year-old woman with rheumatoid arthritis.

The first patient was admitted specifically for the evaluation of the effect of methylandrostenediol upon linear growth and tissue anabolism. Prior to admission, over a period of six months, she had grown 3 inches and had gained 11½ pounds during testosterone therapy. Methylandrostenediol

was administered in Patients MWIL and BAL prior to and/or in conjunction with ACTH and cortisone therapy.

Diet. Throughout the entire balance study each patient received her entire dietary intake by means of a polyethylene tube, the tip of which was placed either in the stomach (in Patient SHE), or in the third portion of the duodenum (in Patients MWIL and BAL). A formula-type diet containing 100 Gm. of protein, 27 Gm. of fat, and 268 Gm. of carbohydrate was administered at hourly intervals throughout the twenty-four hours to each patient, so that a constant intake was assured. Patients SHE and BAL continued this same intake throughout the course of their methylandrostenediol studies, whereas Patient MWIL received additional calories (as fat) late in the study because of weight loss.

Supplementary vitamins, electrolytes and distilled water were administered daily in constant amounts; and the daily activity of each patient remained relatively constant.

Collections. Three-day pooled urines and six-day (or more) pooled stools were collected and analyzed for nitrogen, potassium, phosphorus and sodium. Urinary 17-ketosteroids were also determined.

Sedimentation rates (Wintrobe) were checked at weekly intervals. Daily, or twice weekly, blood was also drawn for eosinophil counts, and blood sugar and lipid determinations. (The latter values will be reported elsewhere.)

Chemical methods. Nitrogen was determined by the Kirk modification of the micro-Kjeldahl procedure (21); sodium and potassium by flame photometry; phosphorus by the method of Fiske and SubbaRow (22); and 17-ketosteroids by the technique of Drekter *et al.* (23).

The data recorded in graphic style appear in Figures 1 through 6 and follow the form for balance studies previously described (24). The scales for nitrogen, potassium and phosphorus are so arranged that their ratio to each other corresponds roughly to the actual proportions of these elements in protoplasm. Thus protein anabolism or catabolism should be reflected proportionately in all three balances (N:K:P: = 15:1:1).

CLINICAL STUDIES

In addition to the balance studies just described, 3 asthenic male patients were followed clinically for prolonged periods of time for gross evidence of anabolic and androgenic effects of oral and parenteral methylandrostenediol as compared to parenteral testosterone propionate: Patient JW, aged 61, with Parkinson's disease; Patient JR, aged 55, with Parkinson's disease; and Patient CA, aged 74, with rheumatoid arthritis. All 3 were weighed daily, and a record kept of the number of erections each week. Because of their situation in a chronic disease ward, no further studies were possible.

Two 1-year-old male Negro infants, (LC and LW), suffering from malnutrition and chronic inanition, were given parenteral methylandrostenediol and subsequently testosterone propionate, in an effort to compare clinically the anabolic and androgenic effects of these two steroids.

Patient NOB, a 26-year-old eunuchoid, whose response to testosterone had been well determined over a period of several years, received methylandrostenediol for one and one-half months.

RESULTS

Metabolic studies

In Figures 1 and 2 are shown the results obtained in the course of long-term balance studies in Patient SHE (diagnosis, panhypopituitarism). Her

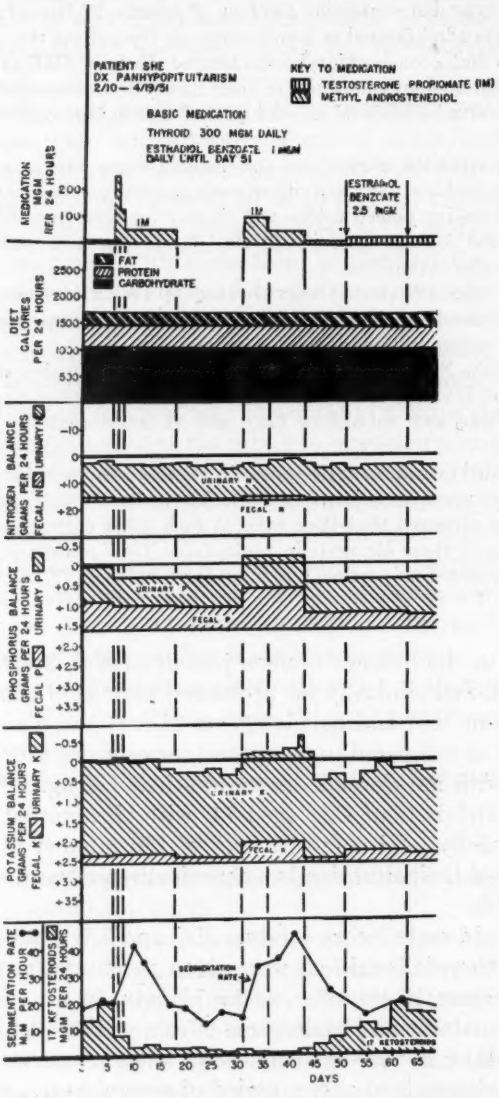

FIG. 1. Metabolic effects of methylandrostenediol in Patient SHE.

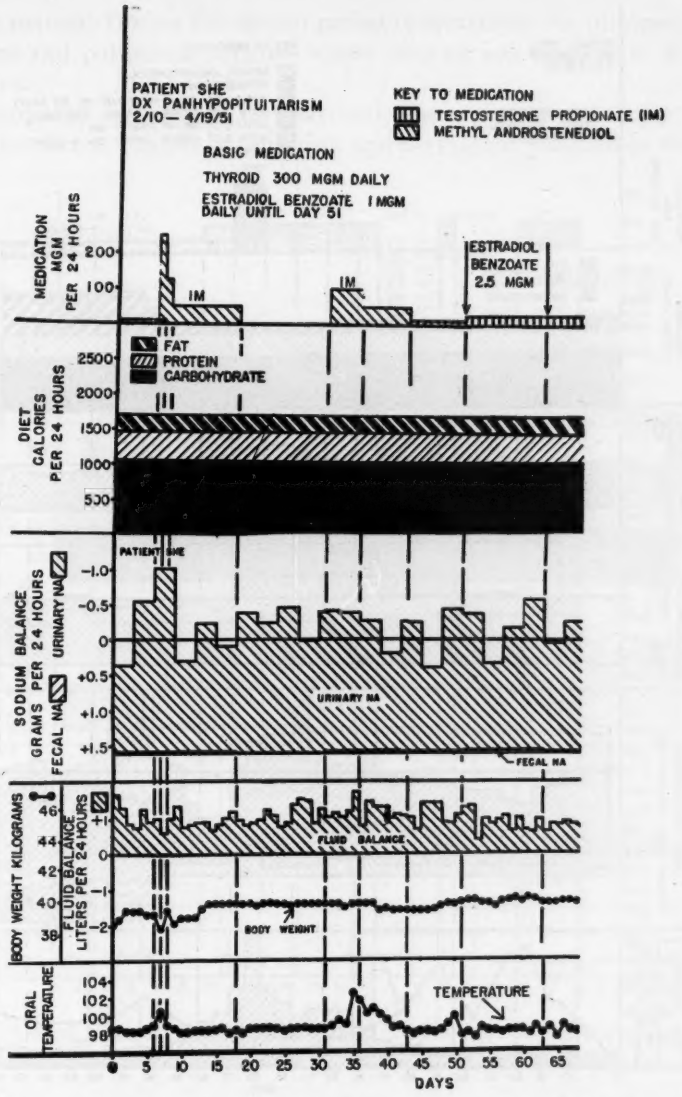

FIG. 2. Effects of methylandrostenediol upon sodium and fluid balance, and upon body temperature in Patient SHE. The "fluid balance" refers to the difference between total fluid intake and urinary output; there is no correction for insensible water loss.

response to methylandrostenediol was probably modified by previous therapy with testosterone. As indicated, this patient received methyl-

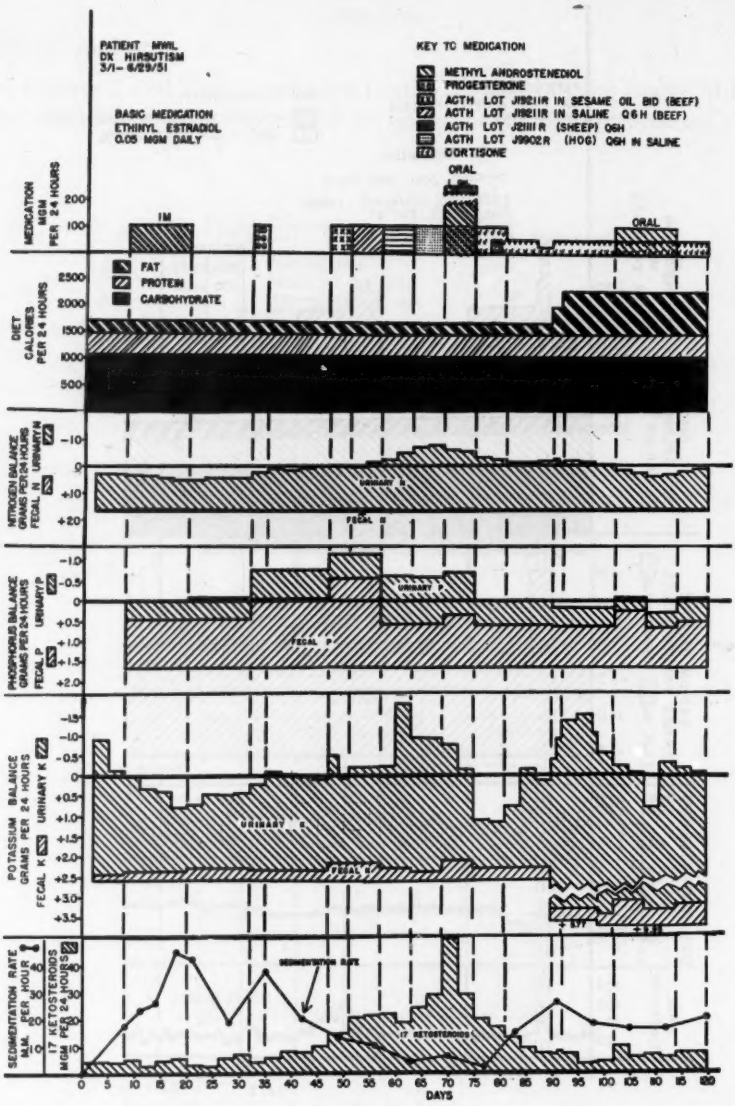

FIG. 3. Effects of methylandrostenediol (alone, and in association with ACTH and cortisone) upon protein balance in Patient MWIL.

androstenediol intramuscularly on two occasions. Except for her first three days of this medication the dosage was either 25 or 50 mg. twice daily. No definite protein anabolic effects from methylandrostenediol were observed

in this patient. During her second period of treatment the nitrogen, phosphorous and potassium balances either became less positive or actually negative.

Throughout each period of treatment, she experienced a fairly severe local reaction at the site of injection and a systemic reaction in terms of

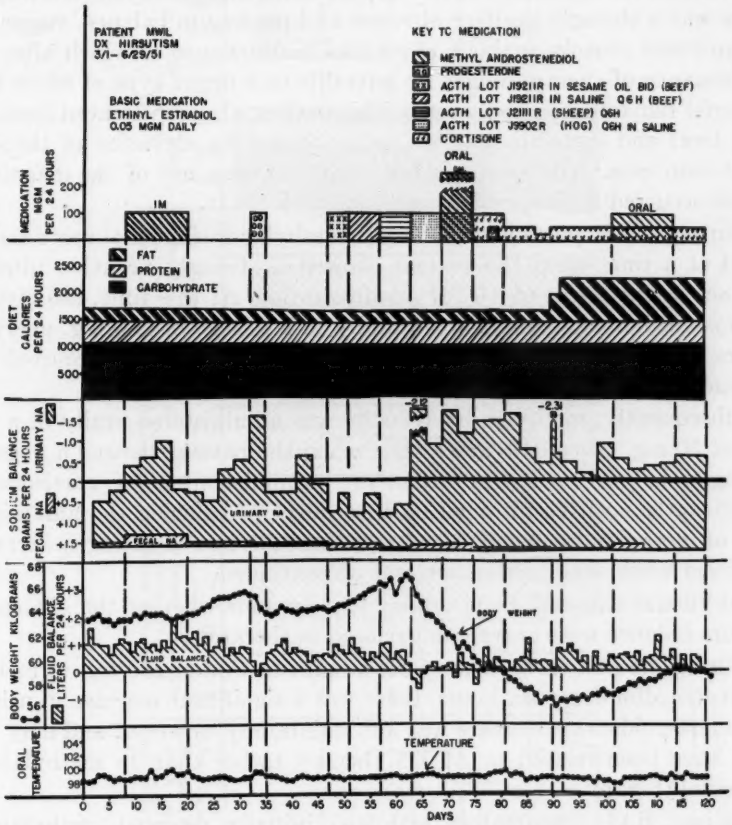

FIG. 4. Changes in sodium, fluid balance, and body weight during administration of methylandrostenediol and other hormonal agents, in Patient MWIL.

fever, malaise and elevation of sedimentation rate. It is probable that the increased nitrogen, potassium and phosphorus excretion during the second period of methylandrostenediol administration is referable to this febrile reaction.

No significant effect was observed upon sodium balance, or upon fluid balance.

17-Ketosteroid excretion during the periods of methylandrostenediol administration was extremely low, indicating that this compound is not excreted as a urinary 17-ketosteroid.

The findings in Patient MWIL are shown in Figures 3 and 4. Initially, this patient received methylandrostenediol, 50 mg. twice daily intramuscularly, for a period of twelve days. During and following such therapy there was a strongly positive nitrogen and potassium balance, suggesting a significant protein anabolic effect, the maintenance of which after discontinuance of therapy might be referable to a depot type of effect from material remaining in the tissues. This patient also experienced considerable local and systemic reaction, as evidenced by elevation of the sedimentation rate.[1] The local reaction, however, was not of the magnitude which occurred in the previous patient.

During a later portion of her study, methylandrostenediol was administered at a time when the patient showed a strongly negative nitrogen balance as the result of ACTH administration. At this time, the methylandrostenediol was administered orally in a dosage of 250 mg. every six hours. A decrease in the excretion of nitrogen and potassium occurred during such administration.

Subsequently, methylandrostenediol was administered orally in a dosage of 50 mg. twice daily, at a time when the patient showed a negative nitrogen balance resulting from the administration of cortisone. A significantly positive nitrogen balance developed during the administration of methylandrostenediol and a gradual increase in nitrogen excretion occurred when such medication was discontinued.

Additional data will be necessary to determine whether the changes in sodium balance were or were not related to therapy.

During the first three days of the administration of 250 mg. of methylandrostenediol every six hours, there was a significant increase in urinary 17-ketosteroids; this increase was not maintained, however, and may very well have been related to ACTH therapy rather than to methylandrostenediol therapy.

Patient BAL (rheumatoid arthritis) initially received methylandrostenediol intramuscularly in a dosage of 25 mg. twice daily and subsequently received the material orally in a dosage of 250 mg. every six hours. Relatively mild local and systemic reactions developed to the parenterally administered material. Figures 5 and 6 show that, except for the apparent

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[1] Two months after these injections a local abscess requiring incision and drainage developed at one of the injection sites. The material aspirated appeared to be methylandrostenediol mixed with purulent exudate.

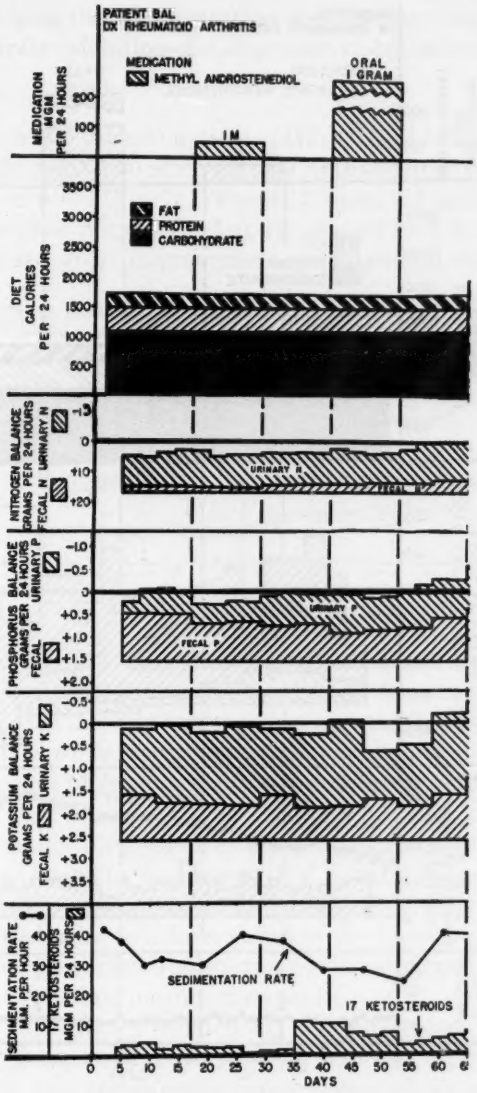

FIG. 5. Effects of methylandrostenediol in Patient BAL.

"rebound" in nitrogen, phosphorus and potassium excretion following discontinuance of orally administered methylandrostenediol, there was little evidence of a protein anabolic effect in this patient. Weight loss also occurred during this period of "rebound."

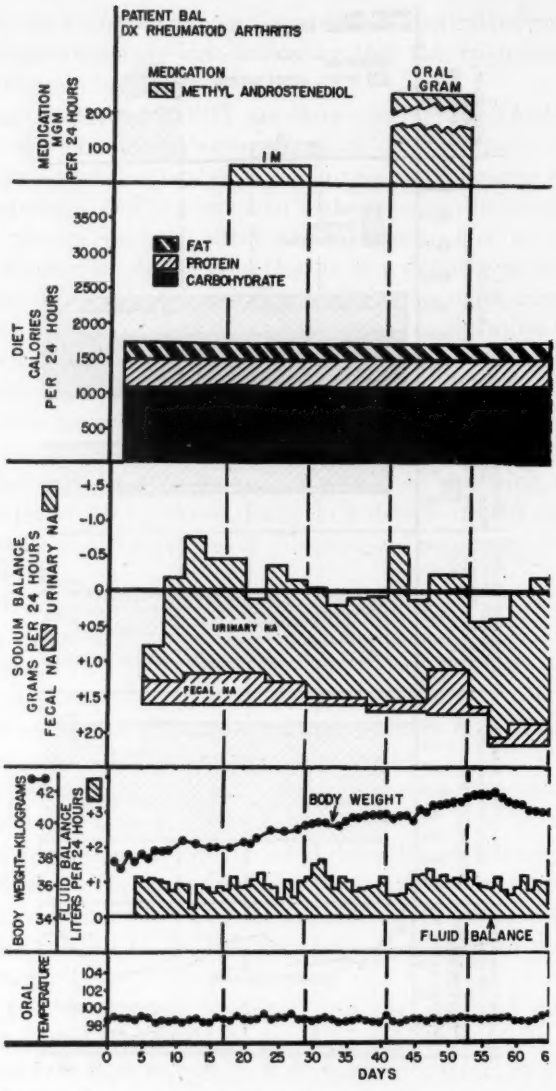

FIG. 6. Effects of methylandrostenediol in Patient BAL.

The increase in 17-ketosteroid excretion in this patient between the two periods of methylandrostenediol therapy may be attributable to the weight gain and improvement in general nutrition, rather than to any specific relationship to hormonal therapy. The decrease in urinary 17-ketosteroids

during and following the administration of 1 Gm. per day of the material orally, may indicate inhibition of endogenous 17-ketosteroid formation.

Clinical studies

Of the 3 chronically ill male patients (JW, JR and CA) none showed an impressive weight gain while receiving oral methylandrostenediol, although slight increases occurred (Fig. 7). Patients JW and CA gained weight while receiving testosterone propionate, but JR showed very little weight gain. JR, however, noted great improvement in his general strength and well

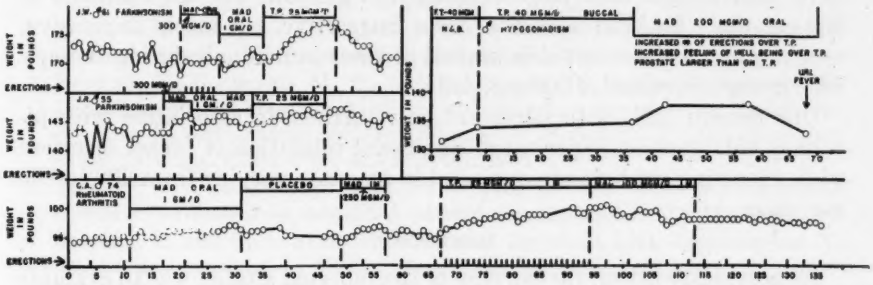

FIG. 7. Clinical studies with methylandrostenediol.

being, and believed that there was a decrease in the symptoms of his Parkinsonism. He received no subjective benefit from methylandrostenediol. Patient JW was the only one to report any significant increase in the number of erections while receiving methylandrostenediol. This may have been because he was physiologically older and manufactured less endogenous androgen than the other 2 men. Toward the end of the study on Patient CA, 50 mg. of methylandrostenediol was given twice daily intramuscularly, in an attempt to prevent the post-testosterone "rebound" described by Albright (25), and seen in Patient JW. A slight effect of this sort may have been achieved. In contrast to the local and systemic reactions to parenteral methylandrostenediol which developed in the 3 young women, Patient CA had no untoward response.

Patient NOB (a pituitary eunuchoid) was given 200 mg. of methylandrostenediol per day orally, after having received various testosterone preparations. The two testosterone preparations which had been given most recently before methylandrostenediol, are shown in Figure 7. He immediately noted an increase in weight, a feeling of well being, and an increased number of erections during methylandrostenediol therapy.

Two malnourished 1-year-old Negro boys (in one, LC, a later diagnosis was pulmonary tuberculosis; in the other, LW, the diagnosis remained incomplete) were treated for fourteen days with methylandrostenediol and

for seven days with testosterone propionate. Neither showed a weight gain on doses up to 50 mg. twice daily intramuscularly of methylandrostenediol, or on 25 mg. daily intramuscularly of testosterone propionate. In one boy (LC), local inflammation developed at the injection sites while he was receiving 50 mg. of methylandrostenediol twice daily. There was no androgenic response in either child during methylandrostenediol therapy, but penile development and frequent erections were observed during the administration of testosterone propionate.

Several other patients with varying degrees of asthenia and malnutrition have received oral methylandrostenediol in amounts varying from 100 to 800 mg. daily for brief periods of time. Suggestive, but rarely impressive, weight gain has been noted in several of these individuals during therapy with dosages in excess of 200 mg. daily.

One patient (JH) with persistent galactorrhea, in association with pituitary enlargement, experienced significant inhibition of breast secretion while receiving methylandrostenediol in amounts varying from 200 to 800 mg. daily.

DISCUSSION

As previously noted, the purpose of the reported studies was to evaluate further the effectiveness of methylandrostenediol as an anabolic agent, and to determine whether, in doses which were sufficient to cause significant growth-promoting effect, an androgenic effect would also be noted.

As the result of the metabolic and clinical studies, the following tentative conclusions were drawn:

1. Parenteral administration of an aqueous suspension of methylandrostenediol causes marked local irritation in many patients.

2. In Patient MWIL parenteral methylandrostenediol produced a significant protein anabolic effect.

3. A dosage of 200 mg. or more per day of orally administered methylandrostenediol may be necessary to obtain a significant anabolic effect.

4. Evaluation of androgenic effects, except on the basis of long-term studies in human subjects, is rather difficult in view of the subjective element involved. It is our impression at the present time that dosages of methylandrostenediol sufficient to produce a significant anabolic effect will also produce as great an androgenic effect as will comparable dosages of testosterone propionate.

5. A dosage of 1 gram of methylandrostenediol daily appears to produce little more anabolic effect than a dosage of 200 mg. daily.

6. On the basis of 1 observation (Patient JH), there appears to be some "pituitary-inhibiting effect" exerted by this compound. The decrease in endogenous 17-ketosteroid excretion in 1 patient (BAL) may also be indicative of such an effect.

SUMMARY

As a result of metabolic and clinical studies, it is tentatively concluded that methylandrostenediol is a relatively weak anabolic steroid. It seems probable that in doses sufficient to produce significant growth-promoting effects, significant androgenic effects also occur. On the basis of these observations, methylandrostenediol offers no clinical advantage as compared to testosterone preparations.

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STUDIES ON THE VALIDITY OF THE HOOKER-FORBES TEST FOR THE DETERMINATION OF PROGESTERONE IN UNTREATED BLOOD*

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IN 1947, Hooker and Forbes (1) described a bioassay for minute amounts of progesterone. The reaction involved changes in the stromal nuclei of the uterus of the castrated mouse and could detect the presence of 0.0002 μg. of progesterone in 0.0006 ml. of test fluid, *i.e.*, 0.3 μg. per ml. In subsequent reports, Hooker and Forbes (2, 3, 4) indicated that the reaction was highly specific and that the hormone circulated in the blood, primarily in the free state, since less than 10 per cent was protein bound. Forbes (5) reported that frequently no bound progesterone could be detected during pregnancy in women and monkeys. Hence it would appear that, for free progesterone, determinations on untreated blood should suffice. This is in contrast with the estrogens, which are approximately 65 per cent protein bound (6, 7). Recently Salhanick, Olsen and Hisaw (8) reported that estradiol possessed the ability to block the action of progesterone on the stromal nuclei of the uterus of the castrated mouse. This could easily invalidate the assay and give erroneous results, particularly in tests which have been carried out on untreated blood samples containing both estrogen and progesterone.

Consequently, the present investigation was undertaken to determine the extent to which the estrogens and other steroids interfere with the Hooker-Forbes assay. In our studies on progesterone levels in the blood of the ewe (9) we obtained results which led us to assume that estrogen was not interfering with the assay. The present results tend to confirm this assumption.

MATERIAL AND METHOD

Adult, female mice of the Rockland-Swiss strain were used in this investigation. The mice were castrated and injected sixteen days later with the test substances. The uterine tissue was fixed in Lawdowsky's fluid and stained with hematoxylin and eosin.

All the hormones were dissolved in sesame oil and injected in a constant volume of 0.0006 ml. The micro-injector was similar to that used by Hooker and Forbes (1). It consisted of a micrometer head mounted on a

Received for publication July 14, 1952.

* Aided by a grant from the Purdue Research Foundation.

metal base to which a 0.25-milliliter tuberculin syringe was attached. This instrument was calibrated, and permitted the accurate delivery of the standard volume of oil. In all instances only one horn of the uterus was injected. Thus no question could arise concerning the interference of different hormones or hormone ratios due to the simultaneous injection of different preparations into the two horns of the uterus. In the antagonism experiments both hormones were dissolved together and injected as a single dose in a 0.0006-ml. volume of oil. The samples were coded, so that subjective interference with the evaluation of the reaction was minimal.

EXPERIMENTAL

Preliminary experiments with our strain of mice indicated a sensitivity comparable to that obtained by Hooker and Forbes (1947). A concentration of 0.3 μg. per ml. gave a positive reaction, whereas 0.1 μg. per ml. failed to influence the stromal nuclei (Table 1). Furthermore, neither estradiol, testosterone propionate, nor pregnanediol[1] gave a positive reaction at extremely high dosages.

TABLE 1. SENSITIVITY TEST FOR PROGESTERONE AND OTHER STEROIDS

Steroid	Concentration	Reaction*
Progesterone	1.0 μg./ml.	++
Progesterone	0.3 μg./ml.	−++++
Progesterone	0.1 μg./ml.	−−−
Testosterone propionate	25.0 mg./ml.	−−
Estradiol	1.0 mg./ml.	−−−
Pregnanediol	0.9 mg./ml.	−

* In this and subsequent tables, each positive or negative reaction represents the response of an individual mouse.

In the first antagonism experiment, 28 mice were used to determine the effect of estradiol on the action of progesterone in the Hooker-Forbes test. The progesterone was injected in two standard concentrations of 2 and 10 μg. per ml., and the estradiol was injected over a concentration range of 1 to 0.01 μg. per ml. of oil. It is obvious from the results that estradiol possesses the ability to block the action of progesterone on the stromal nuclei of the uterus of the castrated mouse. The minimum effective dose of estrogen that inhibited the action of 2 μg. of progesterone was 0.1 μg. of estradiol (Table 2). At lower concentrations the estrogen failed to prevent the action of progesterone, whereas above this concentration, *i.e.*, from 0.1 to 1.0 μg. of estradiol, results of the Hooker-Forbes test were consistently negative. The ratio of estradiol to progesterone that produced

[1] Obtained through the courtesy of Dr. Jaros of Ayerst, McKenna and Harrison, Ltd.

inhibition of the action of the progestogen on the uterus was 1:20, and this same blocking ratio was also obtained with 10 μg. of progesterone. With the latter concentration of progesterone, 0.25 μg. of estradiol failed to block, whereas 0.5 μg. of estradiol produced a definite block. It would appear, then, that the blocking action of estradiol is not dependent on the absolute amount, but on the ratio of the estradiol to the progesterone.

Similar studies were carried out with estrone, involving 32 mice. Again it was observed that the estrogen blocked the action of progesterone (Table 3). A concentration of 2 μg. per ml. of estrone inhibited the action

TABLE 2. ANTAGONISM OF ESTRADIOL AND PROGESTERONE IN THE HOOKER-FORBES TEST

No. of mice	Progesterone, μg. per ml.	Estradiol, μg. per ml.	Ratio of estradiol to progesterone	Results*
4	2.0	1.0	1:2	− − − −
2	2.0	0.2	1:10	− −
3	2.0	0.1	1:20	− − −
3	2.0	0.05	1:40	+ + +
1	2.0	0.02	1:100	+
2	2.0	0.01	1:200	+ +
2	10.0	1.0	1:10	− −
2	10.0	0.5	1:20	− −
5	10.0	0.25	1:40	+ + + + +
2	10.0	0.1	1:100	+ +
2	10.0	0.05	1:200	+ +

* See footnote to Table 1.

TABLE 3. ANTAGONISM OF ESTRONE AND PROGESTERONE IN THE HOOKER-FORBES TEST

No. of mice	Progesterone, μg. per ml.	Estrone, μg. per ml.	Ratio of estrone to progesterone	Results*
6	2	2.0	1:1	− − − − − −
4	2	1.0	1:2	− + + +
4	2	0.4	1:4	+ + + +
2	2	0.2	1:10	+ +
5	10	10.0	1:1	− − − − −
5	10	5.0	1:2	− + + + +
4	10	2.0	1:5	+ + + +
2	20	2.0	1:10	+ +

* See footnote to Table 1.

of 2 μg. of progesterone, whereas 1 μg. of estrone failed to inhibit. Likewise, 10 μg. of estrone antagonized 10 μg. of progesterone, whereas 5 μg. of estrone failed to block the action of 10 μg. of progesterone on the stromal nuclei. It should be emphasized that the ratio of estrone to progesterone is important, and not the absolute amount of either hormone. At a ratio of 1:1 there is sufficient estrone present to antagonize the action of progesterone.

Studies with estriol failed to indicate any antagonism to progesterone (Table 4). Estriol in a concentration ranging from 2 to 20 μg. per ml.

TABLE 4. FAILURE OF ESTRIOL TO BLOCK THE ACTION OF PROGESTERONE ON THE STROMAL NUCLEI OF THE UTERUS OF THE MOUSE

No. of mice	Progesterone, μg. per ml.	Estriol, μg. per ml.	Ratio of estriol to progesterone	Response*
2	2.0	2	1:1	++
2	2.0	4	2:1	++
3	2.0	10	5:1	+++
2	2.0	20	10:1	++
3	1.0	20	20:1	+++
2	0.5	20	40:1	++
4	0.5	30	60:1	++++

* See footnote to Table 1.

did not antagonize 2 μg. of progesterone. When the progesterone concentration was lowered to 1 μg. and subsequently to 0.5 μg. per ml., positive Hooker-Forbes reactions were obtained in spite of the concomitant injection of 20 μg. of estriol. A final test was carried out using 0.5 μg. of progesterone and 30 μg. of estriol per ml. of oil. Again, the estriol, at this ratio of 60 parts of estrogen to 1 part of progesterone, failed to block the action of the progestogen on the stromal nuclei.

Tests for a possible antagonism by pregnanediol and testosterone propionate were also carried out in the same manner. Using a standard concentration of 2 μg. of progesterone per ml. of oil, pregnanediol failed to inhibit the action of progesterone at a dosage ranging from 4 to 500 μg. per ml. of pregnanediol (Table 5). A final test, involving pregnanediol and progesterone at a ratio of 500 to 1, failed to show any antagonism. In a similar manner, testosterone propionate failed to inhibit the action of 2 μg. of progesterone at a dosage ranging from 100 to 1,000 μg.

DISCUSSION

It is obvious from these results that the two estrogens, estradiol and estrone, possess the ability to inhibit the action of progesterone on the

stromal nuclei of the uterus of the castrated mouse. Recently Courrier (10) reviewed the antagonism of estrogen and progesterone and showed that it occurs with a wide variety of receptors. However, the ratio of the two hormones at which inhibition occurs is of fundamental importance and varies with each type of test. Whereas Salhanick *et al.* (8) obtained a block at a ratio of estradiol to progesterone of 1:200, our results indicate a block

TABLE 5. FAILURE OF PREGNANEDIOL OR TESTOSTERONE PROPIONATE TO INTERFERE WITH THE ACTION OF PROGESTERONE ON THE STROMAL NUCLEI OF THE UTERUS OF THE MOUSE

No. of mice	Progesterone, μg. per ml.	Pregnanediol, μg. per ml.	Ratio of pregnanediol to progesterone	Response*
2	2	4	2:1	++
4	2	200	100:1	++++
2	2	500	250:1	++
2	2	500	250:1	++
2	1	500	500:1	++
		Testosterone propionate, μg. per ml.	Ratio of test. prop. to progesterone	
2	2	100	50:1	++
2	2	200	100:1	++
4	2	500	250:1	++++
1	2	500	250=1	+
2	2	1,000	500:1	++

* See footnote to Table 1.

at 1:20. The maximum estrogen concentration reported during pregnancy in the cow and the human being varies from 0.001 to 0.0055 μg. per ml. of blood (11). If it takes 0.1 μg. of estradiol to block 2 μg. of progesterone, it is apparent that there is an insufficient quantity of estrogen present to interfere with the action of progesterone. Furthermore, if we accept the sensitivity of the Hooker-Forbes test at 0.3 μg. of progesterone per ml., as shown originally by Hooker and Forbes (1) and confirmed in this investigation, then the presence of 0.016 μg. per ml. of estradiol would be required. This is approximately twice the maximum concentration of estradiol reported thus far in the blood.

In addition, Roberts and Szego (12) have reported that circulating estrogen is probably estriol, bound to a protein. This estrogen is much less active than estradiol, as has been shown by many investigators, and it has no antagonistic action to progesterone at a ratio of 60 parts of estriol to

one part of progesterone in the Hooker-Forbes test. One would, therefore, expect no interference from estriol in the action of progesterone on the stromal nuclei. Hence we may conclude that, though estradiol and estrone antagonize the action of progesterone, this effect does not interfere with the validity of the Hooker-Forbes assay.

Hisaw and Velardo (13) reported that a number of steroids such as pregnanediol, testosterone, desoxycorticosterone acetate and cortisone possess the ability to prevent the action of progesterone in the development and maintenance of the decidual reaction. Consequently two of these steroids, pregnanediol and testosterone propionate were tested in the Hooker-Forbes reaction. As much as 500 parts of either of these two steroids failed to block the action of 1 part of progesterone.

Recently both Haskins (14) and Butt *et al.* (15) have failed to detect progesterone in the blood of pregnant women. Such an observation certainly casts doubt on whether progesterone as such is the circulating hormone but does not invalidate the bioassay. It would simply indicate that the assay will have to be re-standardized against the "true circulating hormone," when its chemical nature becomes known. In the meantime the Hooker-Forbes test can be used to gain much important information concerning this substance.

Sensitivity studies have indicated that the Swiss strain of mice is as sensitive as the mice used by the original investigators (1). In the present investigation we were able to detect a concentration of 0.3μg. of progesterone per ml. of solution.

SUMMARY AND CONCLUSIONS

It has been shown that the simultaneous, intra-uterine injection of certain estrogenic steroids and progesterone will prevent the action of progesterone on the stromal nuclei of the uterus of the mouse. Estradiol blocked the reaction at a ratio of 1 to 20 and estrone blocked it at a ratio of 1 to 1; estriol failed to block it at a ratio of 60 to 1. The blocking action of the estrogens was shown to depend on the ratio of estrogen to progesterone, and not on the absolute amount.

It was concluded from an analysis of the concentration of estrogen and progesterone in the blood that there was an insufficient amount of estrogen present to interfere with the assay of progesterone. Consequently, we may conclude on the basis of these results that, under normal physiologic conditions, the Hooker-Forbes test is valid for the determination of progesterone in untreated blood.

Tests involving both pregnanediol and testosterone propionate failed to reveal any antagonism to progesterone. Ratios as high as 500 to 1 failed to

interfere with the action of progesterone on the stromal nuclei.

The present results confirm the observation of Hooker and Forbes, that this test will detect a concentration of 0.3 μg. of progesterone per ml.

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INSTRUMENTATION FOR THYROID MEASUREMENT*

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INTRODUCTION

THE problems arising from the use of radioactive iodine, both in the study of thyroid physiology and in its applications to routine thyroid tests, may be roughly divided into three categories. The first is the absolute determination of the quantity of radioactive iodine within a region in the body, *e.g.*, the thyroid gland itself; the second is the determination of the relative distribution of radioactive iodine in certain regions of the body; and the third is the measurement of activity in biologic samples of body fluids. The first of these problems entails the usual difficulties encountered in absolute measurements. It is not only necessary to obtain readings which are statistically significant, but also to assess factors which systematically affect the results. Although the problem of localization of activity within the body does not present many of the difficulties of the absolute determination, one must carefully select a compromise between sensitivity and resolution. The measurement of activity in body fluids presents problems of an entirely different nature. In general, activity in biologic samples may be assayed more readily than activity *in vivo;* but in certain cases, such as measurement of the plasma concentration, the amount of radioactivity may be so small as to make the determination very difficult.

In all of these problems many measuring techniques which are thoroughly satisfactory in skilled hands are found to be very difficult to apply on a routine basis. It is the purpose of this paper to describe instrumentation that has been designed for this application. The needs of each laboratory vary, depending upon the type and number of measurements involved, but the fundamental problems encountered in design of instruments are the same.

ABSOLUTE MEASUREMENT

A number of devices have been described for the determination of the absolute amount of radioiodine in the thyroid gland (1–3). In general, these utilize a single gamma-ray detector located at a fixed distance from

Received for publication June 23, 1952.

* Supported by a grant from the Atomic Energy Commission, by an Institutional Grant to the Massachusetts General Hospital from the American Cancer Society, and by the "HNC" gift of Harvard University.

the surface of the neck. The use of a high sensitivity gamma-ray detector, such as a high sensitivity GM counter or a scintillation counter, allows accurate readings to be obtained following administration of comparatively small doses of radioactive iodine. As with any type of detector, several sources of systematic errors must be considered. The first of these is the uncertainty of the position of the sources within the body, and the second is the effect of scattering and absorption of radiation by the tissues. Freedberg (4) has described a device employing four detectors placed equidistantly on a circle about the neck of the patient. This technique makes the

FIG. 1. Patient in position in center of 4-scintillation counter array.

determination of the exact position of the source unnecessary. An additional advantage is that the effect of scattering is minimized because the radiation scattered from one quadrant is, in general, scattered into another quadrant. It should be pointed out that in most cases accurate positioning of a single counter will give satisfactory results. Indeed extrapolation measurements may be made, as described by Myant, Honour, and Pochin (3), to determine the exact center of the source. The four-quadrant counter is ideally suited for rapid and routine measurements of patients, particularly if repeated measurements on the same patient are required.

In Figure 1 is shown a four-scintillation counter array designed to pro-

vide maximum sensitivity and maximum ease for routine measurement. The scintillation counters consist of sodium iodide crystals in the form of cylinders, 1½ inches in diameter and 1 inch thick, sealed in aluminum cans with a pyrex glass window, in an atmosphere of dry nitrogen. Lucite and mineral oil (Nujol) form an optical path from the crystal to the face of the RCA 5819 photomultiplier tube. Figure 2 is a block diagram of the electronic apparatus. The signals from each of the phototubes pass through a cathode follower pre-amplifier and a variable attenuator before being mixed. The signals are then passed through a linear amplifier, a discriminator, and are then scaled with a conventional scaler.

The patient is seated in a chair of adjustable height with the thyroid ap-

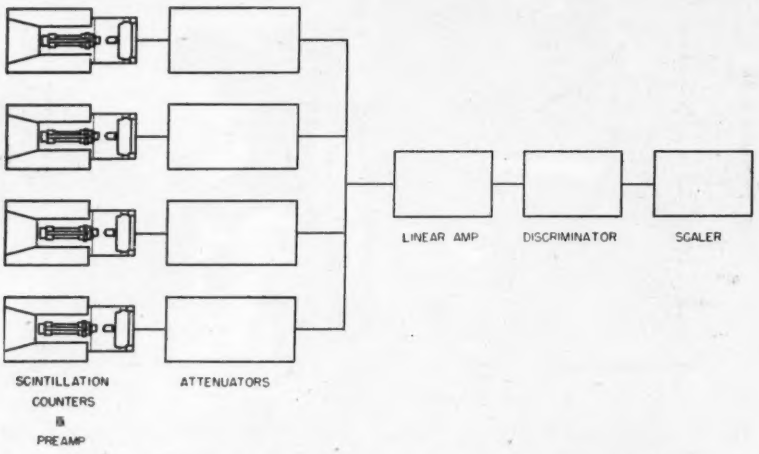

FIG. 2. Block diagram of electronic apparatus for 4-scintillation counter array.

proximately at the level of the four counters. Readings are made at a preset time of one minute. The curves of absolute efficiency versus discrimination level, and background versus discrimination level, are shown in Figure 3. The background rises rapidly in the range of discrimination level of 1 to 5 volts, presumably because of the effect of thermal noise in the phototube. The efficiency curve tends to level off slightly in the range from 10 to 15 volts. Although scintillation counters can be adjusted to give comparatively flat plateaus either against discrimination level or against applied voltage, this slope is not a disadvantage in the present apparatus. The slope of the sensitivity curve of the four detectors allows the matching up of slight differences in the light efficiency of the crystals and the efficiency of the phototube, by means of the variable attenuators. The absolute efficiency as plotted on these curves is the ratio of the number of

counts recorded per unit of time to the number of gamma rays passing through the crystals per unit of time as calculated from the solid angles subtended by the crystals. Because of scattering from the lead collimators placed around the crystals, it is possible that this efficiency is slightly larger than the true absolute efficiency of the crystals. Since lead is a comparatively poor scatterer of soft radiation, this difference is probably small. Sensitivity distribution as a function of position in the region of the four detectors is shown in Figure 4. There is a region of from 15 to 20 cm.

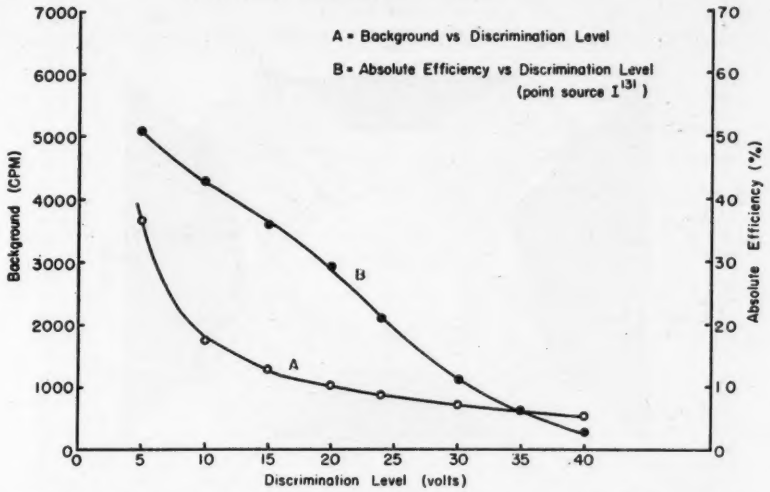

FIG. 3. Background and absolute efficiency as a function of discrimination level.

in diameter in the center of the array, where the counting rate from a point source of radioiodine does not vary by more than ± 5 per cent. The sensitivity increases as the point source is placed near the aperture of each of the detectors and, of course, decreases between the detectors. The vertical sensitivity of the array is seen in Figure 5. The counting rate of the point source in air drops virtually to 0 as the outer edge of the lead collimator is reached.

The effect of scattering and absorption of radiation in the tissues of patients cannot be resolved entirely by physical methods. However, a study of the physical processes which occur is helpful in designing corrective procedures. Figure 6 shows the absolute efficiency of the array for a point source of radioactive iodine located at the center of the array and placed in beakers of water of varying diameters. Curves are plotted of efficiency as a function of diameter for different discriminator settings. These curves

represent a composite of two physical phenomena. The first of these is simple attenuation of the gamma radiation in passing through the absorbing medium. This accounts for the steady decline in sensitivity at larger beaker diameters. The other phenomenon is the production of degraded quanta by the Compton scattering process. Simple scattering of radiation

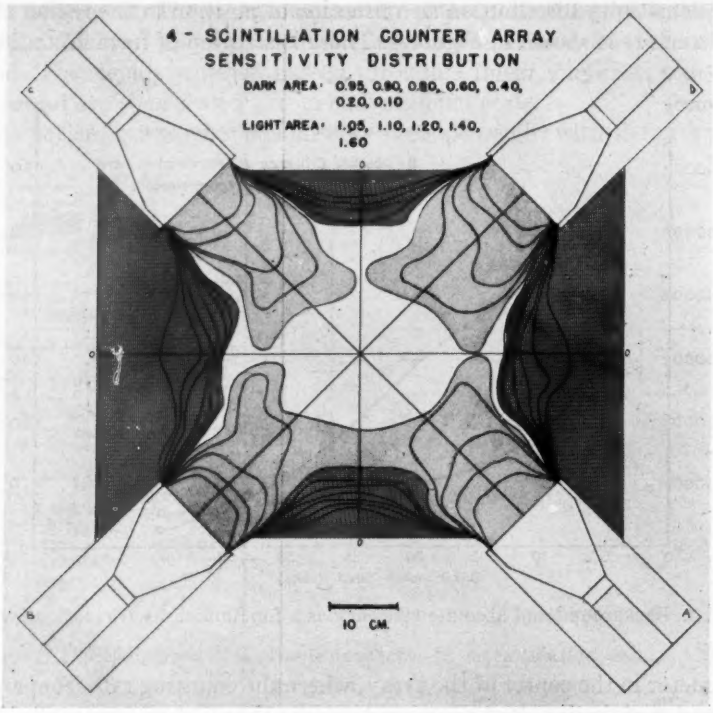
4 - SCINTILLATION COUNTER ARRAY
SENSITIVITY DISTRIBUTION
DARK AREA: 0.95, 0.90, 0.80, 0.60, 0.40, 0.20, 0.10
LIGHT AREA: 1.05, 1.10, 1.20, 1.40, 1.60
10 CM.

FIG. 4. Horizontal sensitivity distribution of 4-scintillation counter array. All sensitivities are relative to unit sensitivity at the center of the array. Iso-sensitivity lines in the light shaded region correspond to 1.05, 1.10, 1.20, 1.40, and 1.60 relative to the center, and those in the dark shaded region, 0.95, 0.90, 0.80, 0.60, 0.40, 0.20, and 0.10 relative to the center.

has no effect on the curves because the source is placed symmetrically at the center of the beaker. The fact that the scattered radiation has lower energy results in a higher efficiency of detection for these quanta. In general, the comparatively high Z sodium iodide crystal stops and records a greater fraction of the lower energy quanta. This effect is more pronounced at the lower discriminator setting because here the pulses of lower energy are recorded.

From a practical point of view, it is not as important to eliminate the effect of scattering and degradation of energy as it is to make this effect independent of neck size and geometry. It will be seen in Figure 6 that the smaller discriminator settings result in a more constant efficiency over the range of beaker diameters corresponding to the dimensions of the neck.

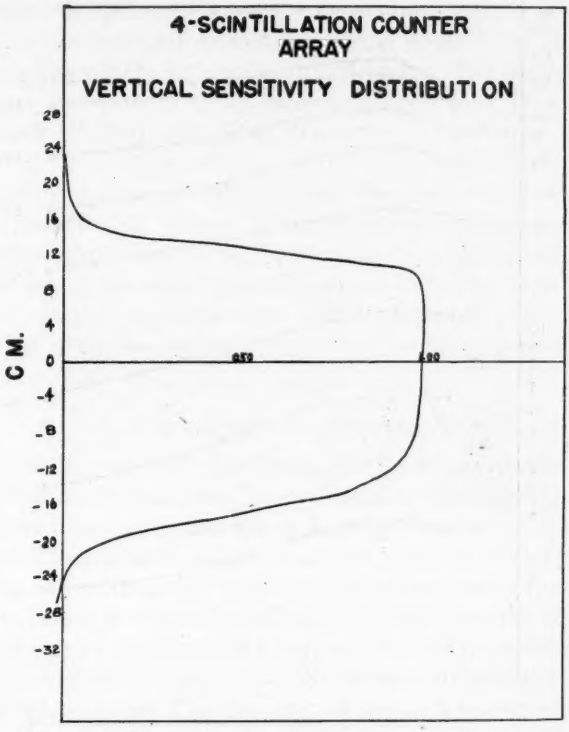

FIG. 5. Vertical sensitivity distribution of 4-scintillation counter array. Sensitivity is plotted relative to unit sensitivity at the mid-point as a function of distance above and below the mid-point on the axis of the array.

However, as decreasing the discriminator settings results in rapidly increasing background rate, the actual discriminator setting must be a compromise between these two factors. In general, the variation over the range of neck diameters is sufficiently small to warrant the use of an average factor to correct for this effect. It should be pointed out that this factor depends not only on the diameter of the neck but also on the physical form of the standard. In the present case, the standard is routinely measured in a 100-cc. volumetric flask. This is indicated in Figure 6 at an ef-

fective diameter of approximately 2 cm. It is seen that there is a definite difference in efficiency of the activity in this flask and as a point source. The exact value of this factor is best determined by using a model more nearly representing the human body. Wax models of the head and shoul-

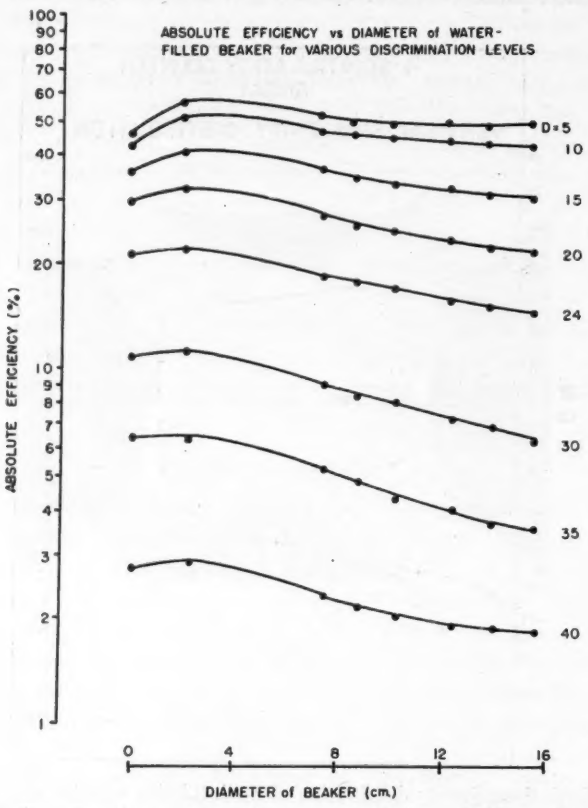

FIG. 6. Absolute efficiency of 4-scintillation counter array as a function of diameter of water-filled beaker surrounding a point source of I^{131} and as a function of discrimination level.

ders have been used to measure this factor, and Myant, Honour, and Pochin (3) have described a technique using a cadaver with radioactive iodine inserted into the thyroid. They have also described measurements made with an ampule of radioactive iodine swallowed by the subject under investigation. For the standardization of the four-counter array, an adequate technique is the measurement of small amounts of radioactive io-

dine placed directly in contact with the neck of the subject. The counting rate is compared with that of the same amount of activity measured in the standard position and the correction factor determined. The standardization of the present apparatus has been achieved by cadaver experiments, determination of radioiodine placed at the surface of the neck, and by comparison of results with those obtained by a single GM counter technique described by Skanse (1).

Tracer doses of radioactive iodine varying from 5 to 20 microcuries have been used routinely for uptake measurement. The technique for preparing tracers has proved quite convenient and may be of interest to others. A stock solution containing 20 microcuries per cubic centimeter is prepared from the original solution of I^{131}. One cubic centimeter of this stock solution is taken for each tracer, over the next two weeks. One standard suffices for all tracers. As an added precaution, each tracer is counted before administering, and the count compared with that of the standard, which is measured several times a day. The sensitivity of the four-counter array is approximately 1,000 counts per minute per microcurie, and that of the background of the four counters is about 900 counts per minute.

DIRECTIONAL COUNTING

The instrumentation which has been used for directional counting is essentially the same as that used for the uptake measurements. Directionality is achieved simply by the use of a lead collimator with an appropriate aperture. The diameter of this aperture may be of any size, but the counting rate will be inversely proportional to the area of the aperture. In most practical cases it is usually sufficient to determine the activity distribution in fairly gross regions of the thyroid or other locations in the body. The apparatus shown in Figure 7, and schematically in Figure 8, has been designed for this purpose. The diameter of the lead aperture is 1 inch, but this diameter may be reduced to ½ inch by means of inserts. An alternative collimator is available which flares outwards, having an opening of approximately 4 inches. This is used for determining the activity in fairly gross regions of the body, for measuring uptakes in patients who cannot be placed in the four-counter array, and for following the time course of uptake by the gland. Figure 8 shows the sensitivity distribution in air for a point source of radioiodine for the 1-inch diameter opening. The sensitivity of this apparatus is such that accurate counts can be obtained on patients who have had the routine tracer dose of radioactive iodine (10 μc). An amplifier with a gain of about 80 is built into the detector so that it can be connected directly to a conventional scaler.

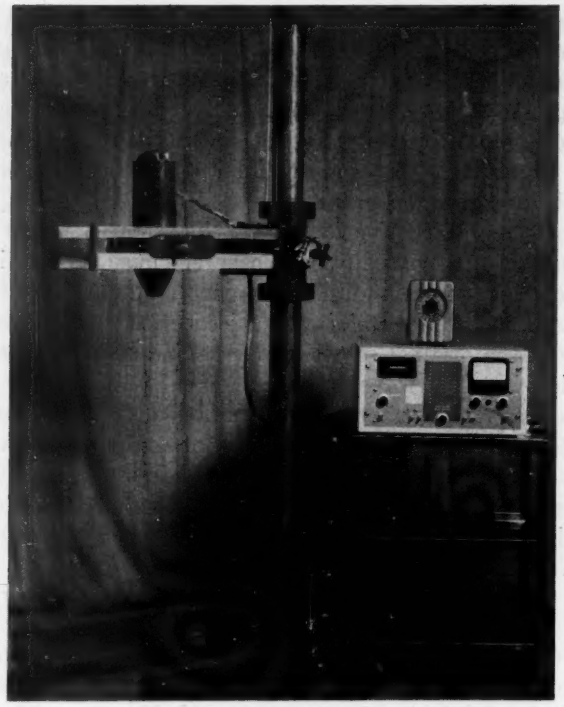

FIG. 7. Directional counter. The position of the detector may be adjusted in three dimensions, and the detector may be rotated about one axis.

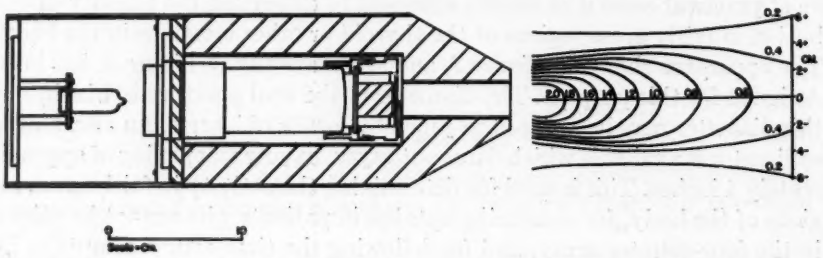

FIG. 8. Schematic diagram of detector and sensitivity distribution of point source of radioiodine, with 1-inch diameter aperture.

ANALYSIS OF BODY FLUIDS

Measurements of radioactivity in urine after the tracer dose of radioactive iodine are usually made by placing the urine flask on a special platform which can be rotated to the center of the four-counter array. A cor-

rection for absorption and scattering must be applied to compare these readings with the readings of the standard flask, but this factor has been found to be independent within wide limits of the volume of urine within the urine flask. The sensitivity of these measurements is approximately the same as that of the *in vivo* determinations, so that activity in 24-hour and 48-hour collections may be readily measured. In the more active urines the radioactivity is measured in a 100-cc. aliquot; or in certain cases, in a few cubic centimeters in a test tube placed adjacent to one of the scintillation counters.

The measurement of radioactive iodine in the plasma at a considerable

TABLE 1. SENSITIVITY OF VARIOUS TECHNIQUES FOR MEASUREMENT OF PLASMA SAMPLES

Techniques	Vol. of Sample (cc.)	Background (counts per min.)	Sensitivity, counts per min. / μc./liter
Planchetting and counting with end-window counters	0.2	42	55
Sample placed adjacent to NaI crystal	2.0	240	64
Iodine distilled from sample and counted as AgI under end-window counter	2.0	28	860
Sample placed in well-type scintillation counter*	2.0	450	2000

* Crystal supplied by Harshaw Chemical Company, Cleveland, Ohio.

time after a moderate tracer dose is probably one of the most difficult problems encountered. For instance, if a dose of 100 microcuries of I^{131} is administered to a patient, at the end of twenty-four days it is probable that the labeled iodine content of the plasma will be as low as .01 per cent per liter. Assuming that a 2-cc. sample of plasma is available for measurement, this means that $\frac{1}{4}$ milli-microcurie of radioactivity is available for measurement, or approximately 10 disintegrations per second in the whole sample. It is obvious that the maximum in sensitivity must be utilized in order to obtain a suitable determination.

Several techniques have been investigated for this purpose. Placing the sample next to a scintillation counter provides suitable sensitivity for most measurements of this type. In addition, it is considerably more convenient than the commonly used technique of planchetting a small sample

of plasma and measuring under an end-window counter. Attempts at ashing larger volumes of plasma have been uniformly unsuccessful because of variable recoveries of activity in the ash. For the samples of lowest activity, the iodine is distilled from the sample, precipitated as silver iodide, filtered, and counted under an end-window counter. The data obtained by three of these techniques are summarized in Table 1. It is assumed that a 2-cc. sample of plasma is available for measurement. Data obtained with a well-type sodium iodide crystal operated as a scintillation counter are also included in the table. This technique would appear to be very useful and convenient for low-level determinations.

SUMMARY

The problems of measurement of radioactive iodine in thyroid study are divided into three parts: absolute measurement, directional counting, and body fluid analysis. The difficulties encountered in each are discussed and typical instrumentation described. It is pointed out that these problems are often not entirely of a physical nature, but may involve physiologic and anatomic considerations.

Acknowledgments

Many people have made suggestions and assisted in the design and testing of the instrumentations described in this report. However, the help and cooperation of many of the members of the Radioactivity Center of the Massachusetts Institute of Technology should be especially mentioned. In particular, Professor Robley D. Evans of M.I.T. has offered invaluable advice and assistance.

Dr. José Barzellato assisted with the iodine extraction technique, and Mr. Robert Cavicchi assisted with much of the mechanical design and assembly.

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A SIMPLIFIED TECHNIQUE FOR THE DIAGNOSIS OF HYPERTHYROIDISM, UTILIZING THE ONE-HOUR UPTAKE OF ORALLY ADMINISTERED I^{131}

KENNETH R. CRISPELL, M.D., WILLIAM PARSON, M.D. AND PHILIP SPRINKLE, B.S.

From the Department of Internal Medicine, University of Virginia, Charlottesville, Virginia

IT IS widely accepted that the 24-hour thyroidal uptake following the oral ingestion of a tracer dose of radioactive iodine (I^{131}) is a useful screening test for hyperthyroidism and hypothyroidism (1, 2).

This study concerns a shortened and simplified screening test for hyperthyroidism, utilizing the one-hour thyroidal uptake following the oral ingestion of a tracer dose of I^{131}.

MATERIALS AND METHODS

One hundred and eighty-two fasting subjects (176 referred for thyroid evaluation and 6 healthy volunteers) were given from 4 to 8 microcuries of radioactive iodine (I^{131}) by mouth. One hour, and again twenty-four hours after ingestion the thyroidal uptake was determined over the gland by means of a scintillation counter,[1] at a distance of 20 centimeters; the result was compared with that obtained on an aliquot of the ingested dose, previously counted at the same distance. The data were expressed as percentage of administered dose.

In addition to the foregoing study, 43 subjects were given a tracer dose of I^{131} (4 to 8 microcuries) intravenously, and the thyroidal uptake was measured at one hour and again at twenty-four hours.

RESULTS

The individual results are shown in Figure 1. Results of the one-hour uptake compared with the 24-hour uptake are shown in Figure 2.

Hyperthyroid vs. Euthyroid—Oral Technique

Thirty-two subjects considered to be hyperthyroid clinically (substantiated by response to therapy) had one-hour uptakes ranging from 20 to 57 per cent. Ninety-six subjects considered to be euthyroid clinically had one-hour uptakes ranging from 7 to 19 per cent. The 24-hour uptake in the two groups ranged from 46 to 98 per cent and from 13 to 46 per cent, respectively.

Two patients, considered to be euthyroid clinically, had both elevated one-hour uptakes (20 and 25 per cent) and elevated 24-hour uptakes (52

Received for publication May 12, 1952.

[1] We are indebted to Dr. Frank Hereford and Mr. Alden Stevenson of the Department of Physics, University of Virginia for the construction of this counter.

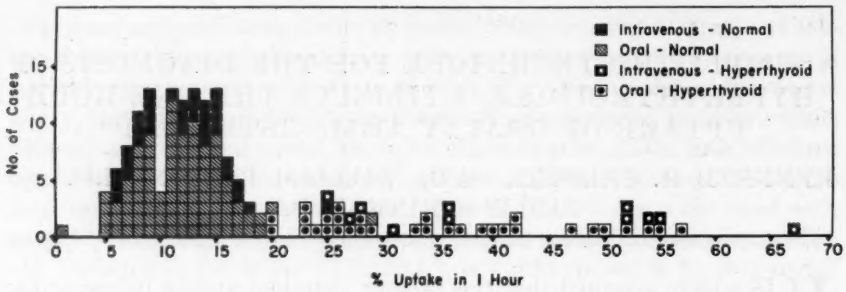

FIG. 1. Comparison of the one-hour thyroidal uptake of I^{131} in normal patients and in patients with hyperthyroidism, using both the oral and intravenous techniques.

and 54 per cent). One of these 2 patients had had a subtotal thyroidectomy, and no pathologic evidence of hyperthyroidism was found; the other is still under observation and has no clinical signs of toxicity.

The administration of thyroid or the administration of iodides, for either diagnostic or therapeutic purposes, depressed both the one-hour and the 24-hour uptakes in both the hyperthyroid and in the euthyroid subjects.

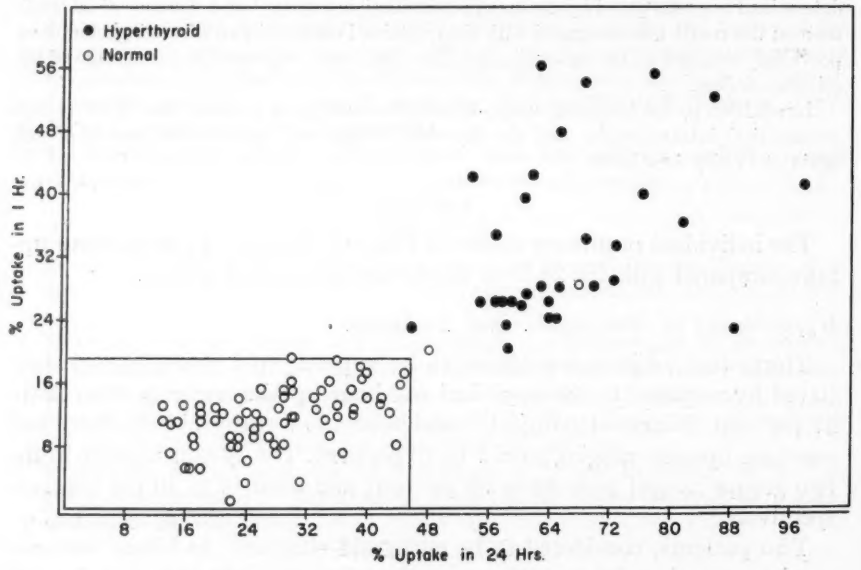

FIG. 2. Comparison of one-hour and 24-hour thyroidal uptakes after oral administration of a tracer dose of I^{131}. The ordinate represents the uptake one hour after administration, and the abscissa represents the uptake at the end of twenty-four hours.

Hypothyroid vs. Euthyroid—Oral Technique

Six subjects with myxedema had one-hour uptakes ranging from 4 to 14 per cent compared with 7 to 19 per cent in the 96 euthyroid subjects. There was a considerable overlap in these two groups.

The 24-hour uptake ranged from 1 to 10 per cent in the myxedema group and from 13 to 46 per cent in the euthyroid group.

Hyperthyroid vs. Euthyroid—I.V. Technique

As shown in Figure 1, results comparable to those with the oral technique were obtained when the intravenous method of administration was used in both euthyroid and hyperthyroid subjects.

DISCUSSION

The results reported indicate that the one-hour thyroidal uptake following the oral administration of a tracer dose of radioactive iodine is as reliable as the 24-hour uptake in separating hyperthyroid from euthyroid subjects.

The 2 subjects, considered to be euthyroid clinically, who had a one-hour uptake in the hyperthyroid range also had a 24-hour uptake in the hyperthyroid range.

In this study there was no instance in which the one-hour and the 24-hour uptakes did not correlate in the differentiation of the hyperthyroid from the euthyroid state.

In differentiating between hypothyroid and euthyroid subjects, there was a considerable overlap of the results when the one-hour uptake was used. The 24-hour uptake, which does give a definite separation, remains the method of choice for this diagnostic problem.

Oral administration gives results similar to those obtained with the intravenous technique originally suggested by Křiss (3).

CONCLUSIONS

1. The thyroidal uptake one hour after the oral administration of a tracer dose of radioactive iodine may be used as a screening procedure to separate the hyperthyroid from the euthyroid state.

2. It compares favorably with the one-hour uptake after intravenous administration and with the 24-hour uptake following oral administration.

3. The technique presented here has the obvious advantages of simplicity of administration and economy of time.

Addendum

While this study was in progress, the following report appeared as a Letter to the

Editor: Morton, M. E., Ottoman, R. E. and Peterson, R. E.: Thyroid uptake measured one hour after small oral doses of radioiodine, *J. Clin. Endocrinol.* **11**: 1572, 1951.

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Letter to the Editor

EVALUATION OF THE HYPERTENSIVE EFFECTS OF NOREPINEPHRINE ON NORMAL AND ADDISONIAN HUMANS

To The Editor:

Recent studies (1, 2) on adrenalectomized dogs have shown that these animals have a distinctly diminished hypertensive response to injected norepinephrine as contrasted to nonadrenalectomized controls similarly treated, suggesting that lack of adequate amounts of cortical steroids reduces the normal response of arteries to physiologic vasoconstrictors (*i.e.*, norepinephrine). In addition, these studies have shown that the initial hypertensive response in adrenalectomized dogs "fatigues" rapidly, whereas in the normal dogs it is well sustained. It was also found that the abnormal responses of adrenalectomized animals could be restored to normal by injections of whole aqueous adrenal cortical extract. Desoxycorticosterone acetate could not restore the normal response.

In the light of these studies, Levine and co-workers postulated that the etiology of shock in adrenal insufficiency was related to the inability of the body to carry out compensatory vasoconstrictive reflexes.

Because of the important theoretical and practical implications of these findings for human pathologic physiology, we felt that it would be desirable to study the effects of injected norepinephrine on normal and on Addisonian humans. This letter records the findings of these studies.

Patients were selected from the wards and private service of the Jewish Hospital of Brooklyn. They were studied in bed, not under basal or fasting conditions. In each case an infusion of 5 per cent glucose in isotonic salt solution was started intravenously, and blood pressures and pulses were recorded every two minutes until a stable level was reached. The infusion was then changed to one containing 4 micrograms per cc. of norepinephrine[1] in 5 per cent glucose in isotonic salt solution, leaving the same needle in situ. Norepinephrine infusions were begun at an initial rate of 6 to 10 drops per minute (1.2 to 2.0 micrograms per minute). Blood pressure and pulse readings were again taken every two minutes until a stable level was reached. The rate of norepinephrine infusion was then increased in each case until a satisfactory hypertensive response was obtained. Fol-

[1] We wish to express our appreciation to Winthrop-Stearns Inc. for their generous clinical supply of Levophed (norepinephrine).

TABLE 1. SUMMARY OF RESULTS DURING INJECTION OF NOREPINEPHRINE

Patient No.	Diagnosis	Resting pulse	Minimum B.P.	Maximum B.P.	Minimum pulse	Pulse decrease	Rise in B.P. on 4γ/min.
1	Addison's dis. (on DCA*)	72	106/58	166/82	68	4	26/16
	Addison's dis. (on cortisone)	84	138/78	168/90	78	6	30/12
2	Addison's dis. (on DCA)	62	116/70	156/86	52	10	20/10
3	Boeck's sarcoid	72	100/60	152/102	56	16	52/40
4	Boeck's sarcoid	74	122/68	176/96	60	14	54/28
5	Chronic osteomyelitis of right femur	84	106/60	160/96	58	26	12/14
6	Hodgkin's disease	84	98/64	124/88	60	24	26/16
7	Bilateral osteoarthritis	86	108/64	146/90	64	22	18/12
8	Rheumatoid arthritis	70	90/50	142/72	56	14	14/10

* DCA = Desoxycorticosterone acetate.

lowing this, the rate of infusion was decreased to the initial level and finally the infusion was discontinued. During the entire study on each patient blood pressures and pulses were taken every two minutes and were allowed to stabilize at each level of infusion rate before the rate was increased or decreased.

Eight patients were studied. Two of these had Addison's disease and before study were being maintained satisfactorily on desoxycorticosterone acetate and supplementary salt. The remaining 6 that served as "normal" controls were hospitalized for the following diseases: 2 had Boeck's sarcoid, 1 had chronic osteomyelitis of the right femur, 1 had Hodgkin's disease, 1 had bilateral osteoarthritis, and 1 had rheumatoid arthritis. None of these patients was acutely ill at the time of study. Patient Number 1 was infused with norepinephrine while on a daily regimen of desoxycorti-

costerone acetate and supplementary salt, and then again while receiving intramuscular cortisone.

A summary of the results may be seen in Table 1. The results of this study indicate that there is no consistent difference in the pattern of response to norepinephrine between normal and Addisonian humans. Certain minor differences between subjects are noted, but no consistent pattern emerges. We did not study the question of "fatiguing" of response because of technical difficulties.

Several reasons suggest themselves for the difference between our results and those of Levine and co-authors: 1) the species difference between dogs and humans, and 2) the possibility that human Addison's disease may not be equivalent to adrenalectomy in animals.

A further consideration is that our normal subjects were not truly normal, but were hospital patients with various chronic diseases. Therefore, it is imperative that further studies be done on healthy normal controls.

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Teaching Clinic

ENDOCRINE THERAPY IN THE MANAGEMENT OF PROSTATIC CANCER

IN THE great majority of patients prostatic cancer presents few diagnostic difficulties because, unfortunately, by the time the patient consults his physician the disease has already extended beyond the borders of the gland and has metastasized to other parts of the body. On the other hand, the diagnosis of early prostatic cancer may not be so easy and many times a biopsy is necessary in order to arrive at a definite decision. It can not be stressed too emphatically that it is important to make a rectal examination at the time of a physical check-up on every male person over 50 years of age.

The etiology of malignant neoplasm of the prostate is unknown and up to ten years ago the treatment of this disease was very discouraging to the physician. However, as a result of the experimental and clinical investigations of Huggins, who discovered that some prostatic cancers are influenced by the presence of androgens in the body, a great improvement in the treatment of this disease has occured. There are still many problems that present themselves in the management of prostatic cancer. This can be well demonstrated in the case of:

Mr. A. B., aged 58, who was first seen in August 1946 because of backache, pain in the hips and legs, and loss of weight and appetite. There was also day and night frequency of urination, with burning. His past history was interesting, in that he had a transurethral resection in May of 1946, because of urinary difficulty. No information was obtained on the histologic examination of the prostate, but he had received no further treatment. About this time he was told that he had silicosis. This diagnosis was apparently made from an x-ray examination of the chest, which revealed that the lungs were filled with multiple discrete nodules.

At the time of his first visit to us he had lost 25 pounds in weight and his appetite was poor. The general physical findings were essentially normal, as was the blood pressure and the results of other routine laboratory procedures. The rectal examination, however, revealed a hard nodular prostate, most certainly carcinoma. A chest x-ray plate showed both lung fields to be filled with multiple rounded areas of increased density, up to 2 cm. in diameter. These were suggestive of extensive metastatic lesions. Further x-ray examination of the genito-urinary system revealed changes in the second and third lumbar vertebrae and pelvis, suggestive of metastases. The kidney outlines were normal in size and position. There were no stone shadows. It was, of course, obvious that the patient had a carcinoma of the prostate with extensive metastases to the soft and bony structures. He was not hospitalized but was given 5 mg. of diethylstilbestrol three times a day. By September 1946, he had gained 15 pounds in weight, his appetite was good, the pains

had disappeared and he had returned to work. By November 1946, he had gained 25 pounds and by December his prostate was normal by rectal palpation.

In April of 1947 the lung fields were interpreted as normal. By this time he had gained 40 pounds and weighed 193 pounds. He had been taking continuous daily doses of 15 mg. of stilbestrol. Because of cramps in the legs and some discomfort of the breast, the stilbestrol was reduced to 1 mg. twice a day. In July 1948, another x-ray examination showed the chest to be normal, as were the findings on physical and rectal examination. Results of routine laboratory tests were within normal limits. He was not seen again until February of 1949. He had been taking 1 mg. of stilbestrol twice a day, except for the previous two months. His examination revealed no unusual findings and he was given 1 mg. of stilbestrol a day. Another x-ray examination of the chest in March 1949 gave negative findings. He was working and free of symptoms. He was not seen again until February 1950. At this time pains had developed in the back, thigh and legs. X-ray plates of the spine, pelvis and femurs showed multiple areas of increased density. By June 1950, he had lost weight and required sedatives for the relief of pain. He stopped taking stilbestrol in February 1950, because his breasts became large, sore and tender. The prostate was normal by rectal examination. Further x-ray plates of the pelvis, femurs and spine showed no change from the previous examination. He was given 0.05 mg. of ethinyl estradiol twice a day. However, no relief of his symptoms occurred and in August 1950 he was hospitalized and a bilateral orchiectomy was performed. Following this he received 1 mg. of stilbestrol twice a day. He shortly had complete relief of his symptoms and returned to work. He was continued on 2 mg. of stilbestrol per day until June 1951. Pains again began to develop in the lower back, legs and groin. X-ray studies of the pelvis, upper end of femurs and lumbar vertebrae showed more extensive changes. Although the lung fields were normal by x-ray studies, metastases had developed in the ribs. The right lobe of the prostate was harder than previously. The excretory pyelograms gave normal results. He now was given a series of x-ray treatments to the pituitary. There was little improvement. He was continued on 1 mg. of stilbestrol twice a day.

In September 1951 the concentration of serum acid phosphatase[1] was 21.5 units per 100 cc. The stilbestrol was increased to 5 mg. a day. By February 1952, he could not work and sedatives were required for the relief of his pain. The serum acid phosphatase level was 26.5 units per 100 cc. and the alkaline phosphatase 9.7 units; the urinary 17-ketosteroids were 6.8 mg./24 hrs. A bilateral adrenalectomy was undertaken but only the right side was completed because of the condition of the patient. He had a stormy convalescense but gradually improved and left the hospital free of pain. Because of the complete relief of pain he refused to have the left adrenal removed.

In September 1952, the serum acid phosphatase level was 46.9 units and the alkaline phosphatase 13.8 units per 100 cc., sodium 143 mEq./L and potassium 4.1 mEq./L. By November 1952 he was having a great deal of pain in the lumbar area, hips, legs and right arm. He returned to the hospital for a left adrenalectomy which was performed on November 21, 1952, without difficulty. It is of interest that the histologic sections of this adrenal gland revealed extensive metastases from the prostatic cancer. Following this, the serum acid phosphatase concentration decreased to 14.2 units per 100 cc. and the alkaline phosphatase to 10.5 units. However, x-rays plates of the chest revealed a recurrence of metastases to the lung fields. His serum sodium and potassium levels were nor-

[1] After the method of Sinowara, G. Y., Jones, L. M. and Reinhart, H. L.: *J. Biol. Chem.* **142**: 921, 1942. Normal values for serum acid phosphatase are 0–2 units per 100 cc.; and for serum alkaline phosphatase, 2.2–8.6 units per 100 cc.

mal, as was his NPN; the blood count and hemoglobin were slightly below normal. There had been some relief of his pain. However, he had not been able to return to work.

The important feature of the endocrine management of this patient was the complete disappearance of metastases to the lungs during the period from 1946 to 1952. The original diagnosis of silicosis was, of course, unfounded. A great improvement was noticed following each form of endocrine therapy, namely, estrogens, castration and adrenalectomy. The longest improvement, however, was from estrogen therapy. It is difficult to explain the relief of symptoms following the removal of the one adrenal. However, this phenomenon has been observed by others. Just how long the present improvement will continue is not known. Although it is now well established that the use of estrogens and/or orchiectomy in the treatment of prostatic cancer is of great value, the merits of bilateral adrenalectomy can not as yet be conclusively appraised. Huggins has recently shown that certain cancers of the breast and prostate do regress for many months and the patients are relieved of their pain.

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Editorial

JOURNAL OF CLINICAL ENDOCRINOLOGY
AND METABOLISM

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THE PITFALLS OF CLINICAL RESEARCH

MANY controversial and contradictory conclusions occur these days in connection with every type of medical research. This is particularly true in the field of clinical endocrinology, a field in which results are unusually hard to evaluate in absolute positive or negative terms. The reasons are probably several.

In the first place there is a habit amongst clinical investigators in this field, probably more than in other specialites, of drawing definite conclusions from the ephemeral and indefinite evidence of symptomatic change. Unfortunately, there is a wide "twilight zone" in the area of truth when positive statements are made on the basis of patients' symptomatic reactions, since these reactions can so easily be interpreted according to the expressed or subconscious desires of the investigator. A good example of this is the familiar and all too frequent type of investigation which reports the effects of therapy upon such conditions as the menopause or dysmenorrhea. The usual technique is a very simple one. Individuals complaining of what the clinician considers to be symptoms of the menopause, for instance, are given treatment with a certain kind of medication and are later questioned as to whether or not these symptoms have been relieved. The procedure frequently acquires the cloak of scientific dignity by the use of a collateral series of controls with a placebo. The question of whether or not the actual symptoms from which the patient is suffering are due to the condition being treated is frequently not gone into very seriously. In this instance the multitudinous symptoms from which patients suffer at the time of the menopause are frequently considered to be due to estrogen deficiency. This may possibly be so, but except for vasomotor instability, there is not a shred of evidence to indicate that it actually is the case. Therefore, when it is stated that relief of these symptoms is due to estrogen replacement, definite conclusions are being made from very in-

definite evidence—certainly a *post hoc ergo propter hoc* type of fallacious reasoning.

A second intellectual hazard for most of us is a great tendency to generalize from specific instances. Herein lies the great danger of drawing conclusions from case reports. An unusual and interesting case is cited wherein, following a certain type of therapy, a patient undergoes a dramatic, spectacular and beneficial change. The implication is obvious that all patients who are suffering from this abnormality should then be given this medication. This type of reasoning occurs very frequently in connection with problems concerning the treatment of sterility, anovulation, and menstrual abnormalities. Everyone who is involved in the investigation and treatment of sterility problems becomes more and more aware as the years go by of the many tangible and intangible variables which occur in this condition. Nothing could be more fallacious than to suggest the general application of a type of therapy because it happened to have been successful in one case. This type of reasoning was proved to have been false about two thousand years ago, and there is no reason why it is not just as false today.

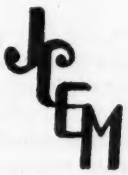
As if these were not sufficient reasons why the literature in connection with endocrine and sterility problems is controversial, there is one other unusual difficulty in connection with this specialty which always must be considered. Whereas, in certain types of medical investigation, the results of animal experimentation can be transferred almost directly to the human, this is not the case with endocrine research. Most animals and humans react very similarly, for instance, in the field of immunology; and investigation of surgical problems can be carried out very advantageously in many animals, even though anatomically they are not necessarily exactly similar to the human. In the field of endocrinology, however, there is an amazing amount of species specificity. The pituitary-ovarian-endometrial relationships, for instance, vary tremendously as one advances up the mammalian scale, from the non-menstruating, easily ovulating lower mammals to the complex, little understood "menstruating anthropoids" and humans. One has only to cite the difference in the effect of chorionic gonadotropin on the ovary of small mammals and of humans to give an indication of this great variability. Many other instances could be cited: the difference in the effect of estrogen upon the pituitary of the rat as compared to its effect in the human; the production of fetal resorption or abortion following excessive estrogen dosage in animals, compared to the present (possibly erroneous but apparently harmless) technique of using excessive estrogen dosage in humans for maintenance of pregnancy. These are but a few examples of the fallacious results which would occur if animal experimentation in this field were applied to the human being.

Many years ago Roger Bacon devised what he called "Four Stumbling Blocks to Truth." They are:

1. "The Influence of Fragile or Unworthy Authority."
2. "Custom."
3. "The Imperfection of Undisciplined Senses."
4. "The Concealment of Ignorance by the Ostentation of Seeming Wisdom.

Possibly, if we clinical research workers review in our minds these and other pitfalls and jealously guard our basic motivation—which is to add to the general fund of useful medical knowledge—there will be less controversial and more truthful material in our medical literature.

C. L. BUXTON, M.D.



Questions and Answers

(Questions submitted at the 1952 Postgraduate Assembly of the Endocrine Society.)

Answers by Norris J. Heckel, Chicago, Ill.

QUESTION: Please discuss briefly the complications and treatment of spermatocele.

ANSWER: Small spermatoceles are quite common in middle aged men, but relatively uncommon before the age of puberty. In the majority of instances they should be left alone because they do not produce any symptoms, nor do complications develop. Occasionally they may reach the size of 6 to 12 cm. in diameter. These usually require removal because of the discomfort produced by the size. Surgical removal of the cyst is superior to other methods of treatment, such as aspiration and injection of various irritants.

QUESTION: Do you always recommend the removal of undescended testes?

ANSWER: The only indications for the removal of an undescended testis are 1) inability to place it into the scrotum, and 2) the presence of a tumor. Both situations are rare, in the reviewer's opinion.

QUESTION: Have estrogens been tried in men with primary testicular failure and vasomotor symptoms?

ANSWER: It is quite possible they have, although no detailed report can be found in the literature. It would seem that estrogens would be contraindicated in such a condition and that androgens would be the treatment of choice.

QUESTION: Many older men, and even middle aged men, who are given testosterone for anabolic effect note decrease in libido and potentia. Please explain.

ANSWER: There might be some argument as to whether the administration of testosterone is indicated in older or middle aged men who do not have an androgen deficiency. In men who do not, the decrease in libido and potentia might be explained on the basis that the administration of testosterone depresses the gonadotropic activity of the pituitary gland.

QUESTION: Is there any evidence that hydrocele fluid contains androgenic hormones?

ANSWER: A recent survey of the literature has failed to uncover any information on the presence or absence of androgens in hydrocele fluid. However, such a report may have been overlooked.

QUESTION: What evidence is there that the testis actually uses FSH?

ANSWER: One of the recent publications that has a bearing on this question is by H. D. Moon, and C. H. Li, in the Proceedings of the Society for Experimental Biology and Medicine (**79:** 505, 1952). These authors notice no effect upon the testis of immature

mice following the injection of FSH. However, in the human it is thought that FSH influences the gametogenic function of the testis.

Answers by Carl G. Heller, Portland, Oregon

QUESTION: What is your opinion in regard to Depo-testosterone?

ANSWER: This question was asked in relation to the evaluation of available androgenic preparations in the correction of permanent testicular deficiencies such as the male climacteric, puberal seminiferous tubule failure associated with failing Leydig-cell function, and functional prepuberal castration. Using economy and convenience as the variable, and equal effectiveness (providing enough androgen is given) as the constant, the following evaluation (listed in descending order of desirability) may be made:

1. Pellet implantation—6 or 7 pellets of testosterone weighing 75 mg. each, given every six months.
2. Intramuscular injection of testosterone propionate (or aqueous suspension), 25 mg. daily or 50 mg. every other day.
3. Sublingual administration of methyltestosterone in divided doses—40 to 80 mg. daily.
4. Oral administration of methyltestosterone, 50–100 mg. daily.
5. Inunction with testosterone.

The questioner elaborated that he meant testosterone cyclopentylpropionate. Under the foregoing circumstances 100 mg. of the "Depo-testosterone" given every three days could be listed in category number 2.

QUESTION: Does chorionic gonadotropin produce gynecomastia? Why? In what percentage of puberal boys?

ANSWER: Chorionic gonadotropin administered in optional amounts (4,000 international units intramuscularly thrice weekly) to boys having delayed adolescence or hypogonadotropic eunuchoidism may cause gynecomastia. The type of gynecomastia is similar in all respects to that encountered in boys undergoing spontaneous puberty. The mechanism for each remains unknown but is presumably the same.

Chorionic gonadotropin administered to normal men or adolescent boys may produce a different type of gynecomastia. The mechanism is as follows: The chorionic gonadotropin acts as an interstitial-cell-stimulating-hormone (ICSH), causing hyperplasia of Leydig cells and associated hypersecretion. The Leydig cells are stimulated to produce about twice the normal amount of androgen, as judged by 17-ketosteroid excretion, and about ten times the normal amount of estrogen. The excessive estrogen production probably causes the observed gynecomastia. (Maddock, W. O., and Nelson, W. O.: New York Academy of Science—Symposium on the Biology of the Testis—1952.)

QUESTION: Would you use APL in the following case? Delayed puberty; male, aged 15; no bodily disproportions; adequate genital size; testes normal in size; small stature; voice unchanged; no sexual hair; one paternal uncle a diabetic.

ANSWER: The "adequate genital size"—if meant to indicate normal for the age, *i.e.*, maturing—strongly suggests that puberty has already been initiated and my prediction is that normal maturation will follow spontaneously. Therefore, there is no necessity, on strictly physiologic grounds, to administer any form of treatment.

Emotional factors, however, would enter into my final decision regarding treatment. If the boy were living under such circumstances that his attention were not drawn to his delay in maturation and no invidious comparisons were being made by his contemporaries, I would decide against treatment. If he were being subjected to such taunts from schoolmates as might be forthcoming in a gymnasium locker-room, which might lead to considerable psychic trauma inasmuch as he might think of himself as only "half a man" or a sexual pervert, then I would favor accelerating maturation. This could be accomplished best by the intramuscular injection of chorionic gonadotropin, 4,000 international units given intramuscularly three times weekly for six weeks only. On the other hand, before such a therapeutic program is entertained, another emotional factor must be taken into account, *i.e.*, in this particular instance, is the psychic trauma attending the knowledge that he needs medical aid to develop into manhood, greater or less great than the psychic trauma occasioned by the delay in maturation?

The Endocrine Society

THE 1953 ANNUAL MEETING

The Thirty-fifth Annual Meeting of The Endocrine Society will be held in the Hotel Statler, New York, N. Y., on Thursday, Friday, and Saturday, May 28, 29 and 30, 1953.

The Committee on Local Arrangements is Dr. Earl T. Engle, Chairman, with Drs. Rulon W. Rawson and Sidney C. Werner as members of the Committee.

All Scientific Sessions will be held in the Hotel Statler. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 29, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Reservations should be made directly with the Hotel Statler, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

The Endocrine Society

1953 AWARDS AND FELLOWSHIPS

Awards and Fellowships presented by The Endocrine Society carry no obligation by the recipient to the Society or to the donors.

Fellowships

Society Fellowships are designed to assist men or women of exceptional promise in furthering their educational training and advancement toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts, in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship will not be awarded in 1953 in order that it may accumulate toward a $5,000 Fellowship to be awarded in 1954.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Laurence Wilson in 1950. Dr. John D. Stoekle was chosen as the recipient of the 1951 Fellowship. Inasmuch as he was unable to accept, due to a call to military service, the same Fellowship was reawarded in 1952 to Dr. C. L. Courtright. The 1953 Schering Fellowship will be in the amount of $5,000 and will henceforth be given in the same amount on alternate years.

Awards

THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH AWARD

This Award of $1,000 was established in 1939 by The Squibb Institute for Medical Research and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; in 1949 to Dr. Herbert M. Evans; in 1950 to Dr. C. N. H. Long; and in 1951 to Dr. J. B. Collip. None was given in 1943. This Award was increased to the amount of $2,500 beginning with the 1952 Award and the recipient was Dr. James H. Means. A special committee of five members of the Society selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishment of an investigator, not more than thirty-five years of age, in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; and in 1951 to Dr. Albert Segaloff. Effective in 1952, the Award was increased from $1,200 to $1,800 and the recipient was Dr. Seymour Lieberman. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 1, 1953*.

The American Goiter Association

1953 ANNUAL MEETING

The 1953 Annual Meeting will be held *May 7, 8 and 9,* in the Drake Hotel, Chicago, Illinois.

VAN METER PRIZE AWARD FOR 1953

The American Goiter Association again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association, which will be held in Chicago, Illinois, May 7, 8 and 9, 1953, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, not later than *February 15, 1953.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association.

BOOKS RECEIVED

American Lecture Series. Springfield, Illinois, Charles C Thomas, Publisher.

Biochemistry of Gastric Acid Secretion. By EDWARD J. CONWAY, M.D., D.Sc., F.R.S., Professor of Biochemistry and Pharmacology, University College, Dublin, Ireland. A Monograph in the Bannerstone Division of American Lectures in Biochemistry and Biophysics, edited by W. Bladergroen, Ph.D., Sandoz Ltd., Basle, Switzerland; 196 pages; 29 figures; 13 tables; 1953.

Body Temperature: Its Changes With Environment, Disease and Therapy. By W. A. SELLE, Ph.D., Professor of Biophysics and Physiology, University of California Medical School, Los Angeles, California. A Monograph in American Lectures in Physical Medicine, edited by W. A. Selle, Ph.D.; 118 pages; 23 figures; 1952.

B-Vitamins for Blood Formation. By THOMAS H. JUKES, Ph.D., Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y. A Monograph in American Lectures in Pharmacology, edited by Chauncey D. Leake, Ph.D., Vice-President in Charge of Medical Affairs, The University of Texas Medical Branch, Galveston, Texas; 124 pages; illustrated; 1952.

Practical Blood Grouping Methods: A Manual of Immunohematology. By ROBERT L. WALL, M.D., Dept. of Research Medicine, The Ohio State University Hospital, Columbus, Ohio. A Monograph in the Bannerstone Division of American Lectures in Hematology, edited by Russell L. Haden, M.D., formerly Chief of the Medical Division, Cleveland Clinic, Cleveland, Ohio; 176 pages; 1952.

Biochemistry of Disease. Edition 2. By MEYER BODANSKY, M.D. and OSCAR BODANSKY, M.D., Ph.D., Professor of Biochemistry, Sloan-Kettering Division, Cornell University Medical College; 1222 pages; illustrated; numerous references; 1952. New York, The Macmillan Company. Price $12.00.

Endocrine Treatment in General Practice. By 21 contributors. Edited by MAX A. GOLDZIEHER, M.D. and JOSEPH W. GOLDZIEHER, M.D., New York; 488 pages, with dietary supplement in addition; 1953. New York, Springer Publishing Company, Inc. Price $8.00.

Gynecology: Diseases and Minor Surgery. By 43 contributors. Compiled and edited by ROBERT J. LOWRIE, M.D., F.A.C.S., D-OG., Associate Clinical Professor of Obstetrics and Gynecology, College of Medicine, New York University; 852 pages ($7\frac{1}{4}\times10\frac{1}{4}$); 775 illustrations (19 in color); 1952. Springfield, Illinois, Charles C Thomas, Publisher. Price $22.50.

Practical Clinical Chemistry. I. Principles. II. Notes on Methods. III. Solutions and Reagents. By NELL F. HOLLINGER, Ph.D., Assistant Professor of Laboratory Practice, University of California, Berkeley, RICHARD K. LANSING, M.P.H., WILLIAM J. HAYES, M.P.H., DOROTHY L. CHANDLER, M.P.H., and ELIZABETH B. AUSTIN, M.P.H., Associates in Public Health, University of California, Berkeley; 1952. The National Press, Millbrae, California. Price $4.50.

Regeneration and Wound-Healing. By A. E. NEEDHAM, M.A., B.Sc., University Demonstrator in Zoology, University of Oxford; 160 pages; 5 figures; 3 tables; 1952. London, Methuen & Co., Ltd. New York, John Wiley & Sons, Inc. Price $1.75.

Research in Endocrinology. By AUGUST A. WERNER, M.D., Assistant Professor of Internal Medicine, St. Louis University School of Medicine, St. Louis, Missouri. A record of Dr. Werner's activities in scientific medicine, with a foreword by PAUL C. REINERT, S.J., President, St. Louis University; 285 pages; 1952. Edited by Al R.

Schmidt, Belleville Daily Advocate, Belleville, Illinois. Address all correspondence to Dr. August A. Werner, 403 Humboldt Building, St. Louis, Missouri.

Sterility. Its Cause and Its Treatment. By J. JAY ROMMER, Ph.G., M.D., Gynecological Staff, Beth Israel Hospital, Newark, N. J.; 427 pages; 259 illustrations; 1952. Charles C Thomas, Publisher, Springfield, Illinois. Price $12.50.

Transactions of Josiah Macy, Jr. Foundation Conferences, published by Josiah Macy, Jr. Foundation Publications, P.O. Box 575, Packanack Lake, N. J.

Adrenal Cortex. Third Conference, November 1951. Edited by ELAINE P. RALLI, Department of Medicine, College of Medicine, New York University, New York: 204 pages; 1952. Price $3.25.

Connective Tissues. Third Conference, February 1952. Edited by CHARLES RAGAN, Associate Professor of Medicine, College of Physicians and Surgeons, Columbia University, New York; 166 pages; 1952. Price $3.50.

Nerve Impulse. Third Conference, March 1952. Edited by H. HOUSTON MERRITT, Professor of Neurology, College of Physicians and Surgeons, Columbia University, New York; 176 pages; 1952. Price $3.50.

Problems of Aging. Fourteenth Conference, September 1951. Edited by Nathan W. SHOCK, Chief, Section on Gerontology, National Heart Institute, National Institutes of Health, and the Baltimore City Hospitals, Baltimore, Maryland; 138 pages; 1952. Price $3.00.

Renal Function. Third Conference, October, 1951. Edited by STANLEY E. BRADLEY, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York; 210 pages; 1952. Price $3.50.

Shock and Circulatory Homeostasis. First Conference, October 1951. Edited by HAROLD D. GREEN, Professor of Physiology and Pharmacology, Bowman-Gray School of Medicine, Wake Forest College, Winston-Salem, N. C.; 245 pages; 24 figures: 9 tables; 1952. Price $3.50.